

# **UNIVERSIDAD COMPLUTENSE DE MADRID**

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## **TESIS DOCTORAL**

**Profiles of brain magnetic activity in normal aging and mild cognitive impairment during resting state and the performance of the paradigm "IDICS"**  
**Perfiles de actividad magnética cerebral en el envejecimiento normal y el deterioro cognitivo leve durante el estado de reposo y la realización del paradigma "IDICS"**

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## **ORIGINAL PUBLICATIONS**

This Thesis is based on the three following articles:

#### Study I

**López ME\***, Cuesta P\*, Garcés P, Castellanos PN, Aurtenetxe S, Bajo R, Marcos A, Delgado ML, Montejo P, López- Pantoja JL, Maestú F, Fernandez A. 2013. **MEG spectral analysis in subtypes of Mild Cognitive Impairment**. AGE. DOI 10.1007/s11357-014-9624-5.

#### Study II

**López M.E.\***, Garcés P.\*, Cuesta P, Castellanos P.N., Aurtenetxe S., Bajo R., Marcos A., Montenegro M., Yubero R., del-Pozo F., Sancho M., Maestú F. **Synchronization during an Internally Directed Cognitive State in healthy aging and Mild Cognitive Impairment. A MEG study**. AGE. DOI 10.1007/s11357-014-9643-2.

#### Study III

**Hypersynchronization in alpha band differs progressive from stable mild cognitive impairment. A MEG study**. López ME\*, Bruña R\*, Aurtenetxe S, Pineda JA, Marcos A, Arrazola J, Reinoso AI, Pedro Montejo, Bajo R, Maestú F. Journal of Neuroscience (Under Review).

#### Other publications:

Pablo Cuesta; Ana Barabash; Sara Aurtenetxe; Pilar Garcés; **María Eugenia López**; Ricardo Bajo; Marcos Llanero-Luque; Inés Ancín; José Antonio Cabranes; Alberto Marcos; Miguel Sancho; Akinori Nakamura; Alberto Fernández. Source Analysis of Spontaneous Magnetoencephalographic Activity in Healthy Aging and Mild Cognitive Impairment: Influence of Apolipoprotein E. Journal of Alzheimer's Disease (Under Review).

Ricardo Bajo; Sandra Pusil; **María Eugenia López**; Leonides Canuet; Ernesto Pereda; Fernando Maestú; Daria Osipova; Eero Pekkonen. Scopolamine effects on brain functional connectivity in elderly subjects: a pharmacological model of Alzheimer's disease. Alzheimer's & Dementia (Under Review).

Pablo Cuesta; Pilar Garcés; Nazareth P. Castellanos; **María Eugenia López**; Sara Aurtenetxe; Ricardo Bajo; José Ángel Pineda-Pardo; Ricardo Bruña; Antonio García Marín; Alberto Marcos; María Luisa Delgado; Ana Barabash; Inés Ancín; José Antonio Cabranes; Akinori Nakamura; Miguel Sancho; Fernando Maestú. Resting state network disruption in healthy and pathological aging. Role of APOE genotype. Brain (Under Review).

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**Synaptic Dysfunction in Mild Cognitive Impairment using Magnetoencephalography-derived Functional Connectivity.** NeuroImage: Clinical (Under Review).

Sara Aurtenetxe, Javier García-Pacios, David del Río, **Maria Eugenia López**, Alberto Marcos, Maria Luisa Delgado, Garikoitz Beitia, Fernando Maestú. **Interference modulates working memory in mild cognitive impairment.** Journal of the International Neuropsychological Society (Under Review).

Garcés P., Vicente R., Wibrál M., Pineda J.A., **López M.E.**, Aurtenetxe S., Marcos A., de Andres, M.E., Yus M., Sancho M., Maestú F., Fernández A. **Brain-wide slowing of spontaneous alpha rhythms in mild cognitive impairment.** Frontiers in Aging Neuroscience 5: 1- 7.

Pineda J, Garcés P, **López ME**, Aurtenetxe S, Cuesta P, Marcos A, Montejo P, Yus M, Tamames JA, del- Pozo F, Becker JT, Maestú F. **White matter damage disorganizes brain functional networks in amnesic Mild Cognitive Impairment.** Brain Connectivity. "In press".

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**María Eugenia López**, Pablo Campo, Irene García- Morales, Antonio Gil-Nagel, Francisco del Pozo, Fernando Maestú. **Role of medial temporal lobe in working and episodic memory during encoding.** In Temporal Lobe: Anatomy, Functions and Pathology (2012). Chapter 6, pp. 119- 140. Nova Science Publishers, Inc.

*\* These authors have contributed equally to this work.*

## **GLOSSARY OF ABBREVIATIONS OF THE MAIN TERMS**

A $\beta$ : Amyloid  $\beta$ -peptide  
AC: Accuracy  
AD: Alzheimer's disease  
ADL: Activities of daily living  
a-md-MCI: amnesic multidomain MCI  
a-sd-MCI: Amnesic single domain MCI  
APOE: Apolipoprotein E  
APs: Amyloid plaques  
ANOVA: Analysis of variance  
BADS: Behavioural Assessment of the Dysexecutive Syndrome  
BNT: Boston Naming Test  
CDR: Clinical Dementia Rate  
COWAT: Controlled Oral Word Association Test  
CRUNCH: Compensation-Related Utilization of Neural Circuits Hypothesis  
CSF: Cerebrospinal fluid  
DMN: Default mode network  
EEG: Electroencephalography  
EOG: Electrooculogram  
FAQ: Functional assessment questionnaire  
FAST: Functional Assessment Staging  
fMRI: Functional magnetic resonance imaging  
FOV: Field of view  
GDS: Global Deterioration Scale  
GDS: Geriatric Depression Scale  
HIS: Hachinski Ischemic Score  
HZ: Hertz  
ICA: Independent component analysis  
ICV: Intracranial volume  
IADL: Instrumental Activities of Daily Living  
IDICS: Internally Directed Cognitive State  
LH: Left hippocampus  
MCI: Mild cognitive impairment  
MEG: Magnetoencephalography  
MMSE: Mini mental state examination  
MRI: Magnetic resonance imaging

MTL: Medial temporal lobe  
na-md-MCI: nonamnesic multidomain MCI  
na-sd-MCI: nonamnesic single domain MCI  
NFTs: Neurofibrillary tangles  
NINCDS – ADRA: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association  
pMCI: progressive MCI  
PET: Positron Emission Tomography  
PFC: Prefrontal cortex  
PLV: Phase Locking Value  
RH: Right hippocampus  
SEN: Sensibility  
SL: Synchronization likelihood  
SPE: Specificity  
sMCI: stable MCI  
SMC: subjective memory complaints  
SQUID: Superconducting Quantum Interference Device  
SPECT: Single-photon emission computed tomography  
STD: Standard deviation  
TMT: Trail Making Test  
tSSS: Temporal extension of Signal-Space Separation  
UCM: Universidad Complutense de Madrid  
UPM: Universidad Politécnica de Madrid  
VOSP: Visual Object and Space Perception Test  
WM: Working memory

## **RESUMEN**

La demencia es una de las enfermedades neurodegenerativas que mayor discapacidad y dependencia genera en la población anciana de todo el mundo. Se estima que el 60-70% de los casos de demencia se deben a la Enfermedad de Alzheimer (EA), para la cual todavía no existe un tratamiento curativo. El impacto a nivel cognitivo, físico, psicológico, social y económico que este trastorno genera sobre el paciente, sus cuidadores y la sociedad en general, lo convierten en una de las prioridades de la comunidad científica a nivel mundial. Por ello, existen numerosas investigaciones centradas en el estudio de los estadios iniciales de esta enfermedad que tratan de arrojar luz sobre cuáles son los factores que predisponen a padecerla, y sobre aquéllos que pueden ayudar a ralentizar o a revertir sus efectos, siendo el fin último conseguir mejorar la calidad de vida de las personas que la sufren.

El deterioro cognitivo leve (DCL) se considera un estado intermedio entre el envejecimiento normal y la demencia, principalmente de tipo Alzheimer. Su investigación resulta esencial para caracterizar las primeras manifestaciones de la enfermedad, que suelen afectar a la memoria episódica, y para poder explorar y pronosticar su evolución. Actualmente, son variadas y muy numerosas las aproximaciones desde las que se está abordando el estudio del DCL, lo que está ayudando a tener una comprensión cada vez más profunda de esta condición patológica y por ende, de la EA. Pese a ello, son muchos los interrogantes que a día de hoy quedan todavía por responder.

Con el objetivo de aportar un granito de arena en el estudio de esta patología, en esta Tesis se han realizado tres experimentos de Magnetoencefalografía (MEG) en los que han participado sujetos mayores sanos y con DCL de tipo amnésico. En todos ellos se ha combinado información neurofisiológica, anatómica y cognitiva, con el propósito de abordar de la forma más global posible los distintos planteamientos realizados.

Todo ello ha sido posible gracias a la colaboración voluntaria de un gran número de personas que han participado en un gran proyecto de envejecimiento (PSI2009-14415-C03-01), del cual hemos formado parte.

A continuación se presenta un resumen de cada uno de los tres experimentos que conforman la presente Tesis.

## **Estudio I.**

### **Estudio del espectro de la actividad oscilatoria cerebral en los subtipos de deterioro cognitivo leve**

Con el fin de estudiar los patrones de actividad magnética oscilatoria en los subtipos de DCL amnésico, un total de 105 sujetos se sometieron a un registro de MEG con ojos cerrados: 36 controles sanos, 33 DCLs de tipo amnésico de dominio único y 36 DCLs de tipo amnésico de dominio múltiple. Los valores de potencia relativa fueron calculados y comparados entre los distintos grupos. A continuación, esos valores de potencia relativa se correlacionaron con las puntuaciones obtenidas en los test neuropsicológicos y con los volúmenes de hipocampo.

No se encontraron diferencias significativas entre los tres grupos en edad, género y nivel educativo, pero sí en el volumen de hipocampo y en el estado cognitivo global medido a través del mini examen cognoscitivo (MMSE). A nivel de actividad cerebral, los dos subtipos de DCL mostraron un incremento de la potencia relativa en las bandas de baja frecuencia (en los rangos de frecuencia de delta y theta) y un decremento de los valores de potencia en las bandas de alta frecuencia (en los rangos de frecuencia de alfa y beta), en comparación con el grupo control. Cabe destacar que se encontraron claras diferencias en los valores de potencia entre los dos tipos de DCL amnésico, pero no en la atrofia hipocampal. Los DCL multidominio mostraron un incremento significativo en los rangos de frecuencia de delta y theta, y un decremento también significativo en los rangos de alfa y beta. Además este patrón estaba correlacionado con la ejecución en los test neuropsicológicos, lo que indica que el subtipo multidominio está asociado no sólo con un mayor enlentecimiento de actividad cerebral, sino también con un estado cognitivo más deteriorado que los DCL de dominio único y los controles sanos. Estos resultados sugieren que los DCL multidominio se caracterizan por tener un perfil de actividad cerebral que está más próximo al observado en la EA. Por tanto, se podría considerar que la probabilidad de conversión a demencia será más alta dentro de este subtipo de DCL.

## Estudio II.

### **Sincronización durante un estado cognitivo internamente dirigido en ancianos sanos y pacientes con Deterioro cognitivo leve. Un estudio mediante magnetoencefalografía**

En este estudio se trataron de determinar los patrones de conectividad funcional que son necesarios durante un estado cognitivo internamente dirigido (“Internally Directed Cognitive State” –IDICS) frente a un estado de reposo, en sujetos normales y con DCL. Esta tarea se diferencia de las que son más comúnmente utilizadas con MEG/electroencefalograma (EEG) porque es necesaria la inhibición de los estímulos externos para poder realizarla, permitiendo el estudio de este mecanismo de control tanto en envejecimiento normal como en el patológico. Para alcanzar este objetivo, se adquirieron las señales MEG de 32 sujetos sanos y 38 sujetos con DCL tanto en estado de reposo con ojos cerrados, como durante la realización de una tarea de substracción mental con dos niveles de dificultad.

No se encontraron diferencias en edad, género o nivel educativo, aunque ambos grupos diferían en las puntuaciones del MMSE y el volumen de hipocampo. En el estado de reposo, los DCLs mostraron una mayor conectividad en bandas de baja frecuencia (delta y theta) y una menor conectividad en bandas de alta frecuencia (alfa, beta y gama), que los controles. Estos patrones de conectividad también estaban relacionados con un peor estado cognitivo, lo que sugiere que el DCL podría ser el inicio de un síndrome de alteración de la conectividad funcional con el cual se caracteriza a la enfermedad de Alzheimer en sus fases iniciales. Por otro lado, ambos grupos realizaron la tarea con éxito, aunque a nivel neurofisiológico, los pacientes con DCL mostraron mayores cambios de conectividad entre el estado de reposo y la tarea, que los sujetos controles, lo cual correlacionaba con un peor estado cognitivo. En concreto, en los DCLs se encontró una hipersincronización en las bandas delta, theta, beta y gamma, lo que revela un funcionamiento anómalo en este grupo. Por el contrario, los DCLs mostraron una falta de sincronización en la banda alfa, lo que podría sugerir que sufren un déficit en el control inhibitorio. Además, la magnitud de los cambios de conectividad con el incremento de la dificultad de la tarea en las bandas delta, beta y gamma en el grupo control, y la ausencia de este efecto en el grupo de pacientes, estaría en la línea del modelo CRUNCH (Compensation-Related Utilization of Neural Circuits Hypothesis).



### **Estudio III.**

#### **La hipersincronización en la banda alfa diferencia el Deterioro progresivo del estable. Un estudio de magnetoencefalografía**

Con el objetivo de estudiar si existían diferencias de conectividad funcional entre los pacientes con DCL que convertían a Alzheimer frente a los que permanecían estables, se compararon los registros MEG con ojos cerrados de 30 DCLs estables (DCLe) y 19 DCLs progresivos (DCLp). El tiempo medio de conversión de los DCLp se situaba en el año, por lo que fueron considerados conversores rápidos. Ambos grupos no diferían en edad, en género ni en nivel educativo. Se estudió la conectividad funcional en el espacio de las fuentes en ambos grupos.

Posteriormente, las diferencias significativas encontradas en la comparación de los dos tipos de DCL, fueron correlacionados con distintas medidas neuropsicológicas y con el volumen del hipocampo. Los DCLp en comparación con los DCLs, obtuvieron peores puntuaciones en test de memoria episódica y semántica, y también de función ejecutiva. A nivel estructural no se encontraron diferencias en el volumen de hipocampo entre ambos grupos. Sin embargo, a nivel de conectividad funcional, se encontraron diferencias significativas en 5 links en la banda alfa, entre el cíngulo anterior derecho y el córtex temporo-occipital, principalmente derecho. Los DCLp mostraron un incremento de sincronización entre esas áreas, que además estaba inversamente relacionado con la ejecución en varias tareas cognitivas y con el volumen de ambos hipocampos. Estos resultados sugieren que la pérdida de conexiones gabaérgicas que están normalmente asociadas con la deposición de amiloide, podrían explicar las alteraciones encontradas en esta red.

## **SUMMARY**

Dementia is one of the neurodegenerative diseases that produce the greater disability and dependency in the elderly population worldwide. It is estimated that 60-70% of cases of dementia are due to Alzheimer's disease (AD), for which there is still no cure. The impact of cognitive, physical, psychological, social and economic level that this upset has on the patient, their caregivers and the general society make it a priority for the scientific community worldwide. Therefore, there are many research focused on the study of the initial stages of this disease that try to shed light on the factors that predispose to undergo it, and those which can help to slow or reverse its effects, being their ultimate goal to improve the quality of life of those people who suffer it.

Mild cognitive impairment (MCI) is considered an intermediate state between normal aging and dementia, mainly Alzheimer's type. Its research is essential to characterize the first manifestations of the disease, which usually affect the episodic memory, and to explore and predict its evolution. Currently, there are varied and numerous approaches from being addressed the study of MCI, which is helping to have an increasingly deep understanding of this pathological condition and therefore the EA. Nevertheless, many questions that today there are still unanswered.

In order to provide a bit in the study of this disease, in this Thesis have been conducted three experiments magnetoencephalography (MEG) in which amnesic MCI and healthy elderly subjects have participated. All of them combined neurophysiological, anatomical and cognitive information, in order to address the more global as possible the different approaches made. This has been made possible by the voluntary cooperation of a large number of people involved in a great project of aging (PSI2009 -14415 -C03- 01), which we have been a part. A summary of each of the three experiments that make up this thesis is presented below.

## **Study I.**

### **MEG spectral analysis in subtypes of Mild Cognitive Impairment**

In order to study the patterns of oscillatory magnetic activity in amnesic MCI subtypes, a total of 105 subjects underwent an eyes-closed resting-state Magnetoencephalographic (MEG) recording: 36 healthy controls, 33 amnesic single domain MCIs (a-sd-MCI), and 36 amnesic multidomain MCIs (a-md-MCI). Relative power values were calculated and compared among groups. Subsequently, relative power values were correlated with neuropsychological tests scores and hippocampal volumes.

No significant differences in age, gender and educational level were found among groups, but in hippocampal volume and global cognitive state measured by MMSE. At activity brain level, both MCI subgroups showed an increase of relative power in lower frequency bands (delta and theta frequency ranges) and a decreased of power values in higher frequency bands (alpha and beta frequency ranges), as compared with the control group.

More importantly, clear differences emerged from the comparison between the two amnesic MCI subtypes in power values, but not in hippocampal volumes. The a-md-MCI group showed a significant power increase within delta and theta ranges, and reduced relative power within alpha and beta ranges. Such pattern correlated with the neuropsychological performance indicating that the a-md-MCI subtype is associated not only with a “slowing” of the spectrum but also with a poorer cognitive status than a-sd-MCI patients and controls. These results suggest that a-md-MCI patients are characterized by a brain activity profile that is closer to that observed in Alzheimer’s disease. Therefore, it might be hypothesized that the likelihood of conversion to dementia would be higher within this subtype.

## Study II

### **Synchronization during an Internally Directed Cognitive State in healthy aging and Mild Cognitive Impairment. A MEG study.**

We aimed to determine the functional connectivity pattern required to deal with an Internally Directed Cognitive State (IDICS) in comparison to a resting state, in healthy aging and MCI. This task differs from the most commonly employed in MEG/electroencephalography (EEG) since inhibition from external stimuli is needed to perform it, and it allows the study of this control mechanism in healthy and pathological aging. To this end, MEG signals were acquired from 32 healthy individuals and 38 MCI patients, both in resting state and while performing a subtraction task of two levels of difficulty.

No statistical differences were found between controls and MCIs in age, sex or educational level, but both groups did differ in MMSE and hippocampal volume. In the resting state, the DCLs showed increased connectivity in low frequency bands (delta and theta) and decreased connectivity in high frequency bands (alpha, beta and gamma), than controls. These patterns of connectivity were also associated with worse cognitive status, which suggest that MCI could be the start of disconnection syndrome which characterizes Alzheimer's disease. Furthermore, both groups performed the task successfully, although at a neurophysiological level, MCI patients presented higher connectivity changes between resting and task than those in the control group, and this was related to a lower cognitive performance. In particular, in MCIs a hypersynchronization in delta, theta, beta and gamma bands was found, which reveals an abnormal functioning in this group. Contrary to controls, MCIs presented a lack of synchronization in the alpha band, which may denote an inhibition deficit. Additionally, the magnitude of connectivity changes rose with the task difficulty in delta, beta and gamma bands in controls but not in MCIs, would be in line with the CRUNCH model (Compensation-Related Utilization of Neural Circuits Hypothesis).

### **Study III.**

#### **Hypersynchronization in alpha band differs progressive from stable mild cognitive impairment. A MEG study**

In order to examine whether there were differences in functional connectivity between patients with MCI who converted to AD versus those who remained stable, MEG eyes- closed recordings from 30 stable MCIs (sMCI) and 19 progressive MCIs (pMCI) were compared. The average conversion time of pMCI was of one year, so they were considered fast converters. Both groups did not differ in age, gender or education level. Functional connectivity was studied in source space in both groups.

Then the significant differences between both groups were correlated with neuropsychological scores and hippocampal volume measures. pMCIs obtained poorer scores in episodic and semantic memory and also in cognitive functioning. At structural level, there were not differences in hippocampal volume between both groups. However, at functional connectivity level, 5 significant links in the alpha band between the right anterior cingulate and temporo-occipital cortex, mainly of the right hemisphere, were found. pMCI patients showed an increase in synchronization in these brain areas, which was inversely correlated with the performance in several cognitive tasks and with both hippocampal volumes. These results suggest that the loss of gabaergic synapses usually associated with amyloid deposition could explain the network alterations reported in the present work.

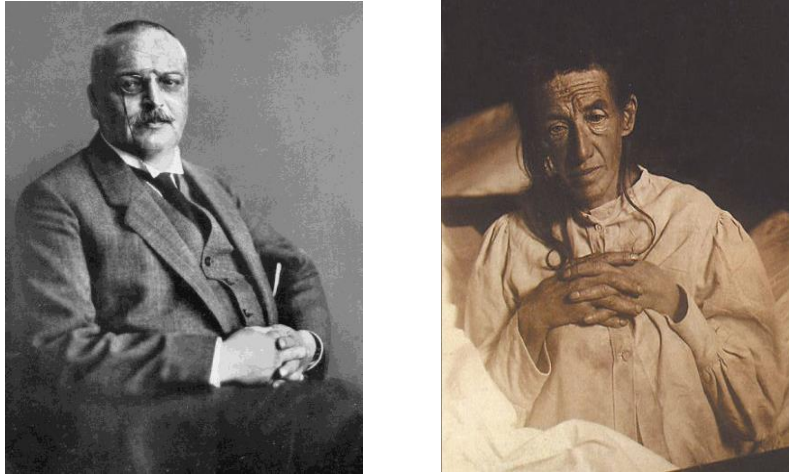
## **GENERAL INTRODUCTION**

## 1. ALZHEIMER'S DISEASE

Dementia is one of the most important pathologies among the elderly population, being Alzheimer's Disease (AD) the most frequency subtype. It affects about 26 million people worldwide, and its prevalence will quadruple by 2050 (Selkoe, 1991). Life expectancy is increasing in most parts of the world, especially in western countries (Blennow, de Leon, & Zetterberg, 2006). This fact increments the risk of its appearance, since the incidence and prevalence of AD start to rise around 65 years and at age of 80 and 90 it is nearly 50% (Zamrini et al., 2011).

AD is a progressive, incurable and neurodegenerative disease whose first cognitive deficit is memory (Corey-Bloom, 2002), and finally produces a devastating global cognitive impairment that results in aphasia, apraxia, agnosia, and impaired in judgment, decision-making, behavior and attention (Hodges, 2006). The first neuropathological case of a patient affected by AD, Auguste Deter, was described in 1906 by Alois Alzheimer. This patient presented a combination of cognitive deficits, psychiatric symptoms, and macroscopic and microscopic brain lesions: amyloid or senile plaques (APs), neurofibrillary tangles (NFTs) and brain atrophy (Alzheimer, 1907). APs are extracellular aggregates of amyloid  $\beta$ -peptide ( $A\beta$ ) in the form of  $\beta$ -plated which accumulate principally in the cortex, and NFTs are mainly composed by a cytoskeletal microtubule-associated protein, called tau, that becomes hyperphosphorylated, dissociates from microtubules, and self-aggregates in the cytosol to form paired helical filaments (Catricala, Torti, & Ricevuti, 2012). Tangles are usually found in limbic structures, such as hippocampal and parahippocampal regions (Price et al., 2001). Related to these two major hallmarks of AD, there is a diffuse neuronal and synaptic loss which produces a reduced brain weight, cortical atrophy and ventricular enlargement. Besides cortical alterations, which occur mainly in temporal and parietal areas, subcortical neuronal loss in the Nucleus basalis of Meynert and in the Locus Coeruleus have been also described. The damage in these nuclei produces a reduction of cholinergic and noradrenergic levels, respectively (Nestor, Scheltens, & Hodges, 2004).





**Figure 1.** On the left, Alois Alzheimer (1864- 1915). On the right, Auguste Deter, the first person diagnosed with Alzheimer's Disease (1849- 1906)

In 1984 the National Institute of Neurological and Communicative Disorders and Stroke- Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) conceptualized the AD as a "dual clinicopathological entity": On one hand patients should present a progressive dementia that included as the primary feature the impairment in episodic memory, and then the deficit in other cognitive functions, being this affectation so severe to interfere with daily living; and on the other hand, patients should present neuropathological alterations, as described above. As neurophysiological changes cannot be measured during life, AD diagnostic was predominantly made based on clinical entity and could only be made in terms of probability as "probable AD" (when there were not comorbid conditions such as depression), "possible AD" (if comorbid conditions were present) while patients were alive. The "definitive AD" diagnosis was made after post-mortem histopathological confirmation (G. McKhann et al., 1984).

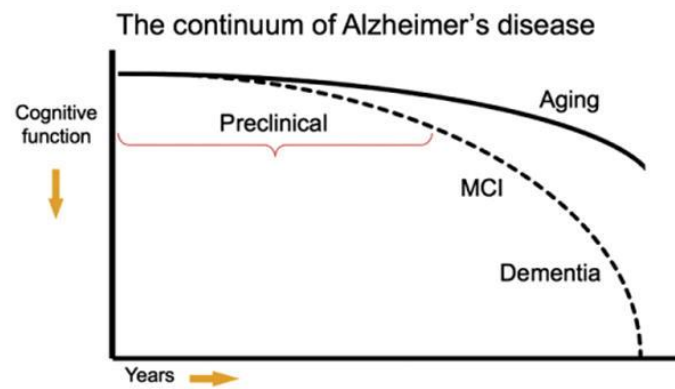
In order to solve this diagnostic uncertainty, in 2007 the International Working Group for New Research Criteria for the Diagnosis of AD proposed a new diagnostic framework (Dubois et al., 2007), based both clinical evidence and in- vivo biomarkers. These biological markers can be measured using magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) and positron emission tomography (PET), and can be divided in pathophysiological markers (reduced concentrations of amyloid  $\beta$  and increased in total tau and phosphorylated tau) and topographical markers (an atrophy of the medial temporal lobe and a reduced glucose metabolism in temporo-parietal regions). The specificity and sensitivity of these biomarkers for Alzheimer's pathology varies, and also their combination.

According to these new criteria, "probable AD" should be replaced with "typical AD" (clinical features are further supported by one or more in vivo positive biomarkers of AD), "possible

AD” by “atypical AD” (the clinical syndromes are non-amnestic, such as primary progressive non- fluent aphasia, logopenic aphasia, posterior cortical atrophy and frontal variant of AD; and a positive biomarker of AD is needed) and “definitive AD” by “neuropathologically verified AD”.

Although this new approach based on the biomarkers has a great potential in the diagnosis of AD, is hardly implemented in the clinical context due to its high cost and also because it is still not enough evidence of its real diagnostic power (Hampel et al., 2010; Fletcher et al., 2013).

Despite all the efforts in the early detection and accurate diagnosis of AD, numerous crucial questions remain unanswered. For example, there is an imperfect relation between biological measures of brain’s pathology, measured cognitive function and real life performance as it has been found that up to 30% of subjects without clinical pathology at autopsy have a pathological profile consistent with AD diagnosis (Riley, Snowden, & Markesbery, 2002; Knopman et al., 2003; G. M. McKhann et al., 2011). These findings are in line with those that suggest that AD begins up to 20 years before disease manifests clinically (Grady et al., 1988; Gray 1994) and hence, it is essential to focus on previous stages of the pathology in order to initiate a treatment aimed on one hand, to improve or prolong patient’s functions and provide independence and quality of life and on the other hand, to increase the effectiveness of current drugs as their effects are more powerful in the initial stages of the disease (Cummings, 2004). Since the prevention is crucial in this neurodegenerative disorder, it is essential to advance in the understanding of the “presymptomatic” or “preclinical” stages of AD. For this reason, Sperling et al. (2011) proposed a model of dementia as continuum, which includes those individuals who present early AD pathological changes but do not meet clinical criteria for MCI or dementia. Within this preclinical phase, we would find subjects who carry one or more apolipoprotein E (APOE)  $\epsilon 4$  alleles, since it is known that they have an increased risk of developing AD; those who are carriers of autosomal dominant mutations or subjects with subjective memory complaints (SMC), who report memory-related complaints that are otherwise undetectable with standardized objective tests of cognitive performance. Subsequently, we would find individuals with Mild cognitive impairment (MCI), which is the main topic of the present Thesis, and finally those who suffer dementia.



**Figure 2.** Model of the clinical trajectory of Alzheimer's disease (AD). The stage of preclinical AD precedes mild cognitive impairment (MCI) and encompasses the spectrum of presymptomatic autosomal dominant mutation carriers, asymptomatic biomarker-positive older individuals at risk for progression to MCI due to AD and AD dementia, as well as biomarker-positive individuals who have demonstrated subtle decline from their own baseline that exceeds that expected in typical aging, but would not yet meet criteria for MCI. Note that this diagram represents a hypothetical model for the pathological-clinical continuum of AD but does not imply that all individuals with biomarker evidence of AD-pathophysiological process will progress to the clinical phases of the illness. Source: Sperling et al (2011).

## 2. MILD COGNITIVE IMPAIRMENT

Mild cognitive impairment (MCI) has been described as a transitional stage between normal aging and dementia (R C Petersen, 2004). It is characterized by objective evidence of cognitive decline that is greater than expected for age and educational level but is still not severe enough to be considered a full-blown dementia as does not interfere with activities of daily living (Petersen et al. 2001; Jelic et al. 1996). Prevalence of MCI ranges from 3 to 19% in the elderly population, showing an incidence from 8 to 58 per 1000 each year (Gauthier et al., 2006).

<b>MCI Diagnostic Criteria</b>	
1. Subjective cognitive complaint, preferably corroborated by an informant	
2. Abnormal function for age in one or more domains of cognitive function	
3. Evidence of decline in one or more aspects of cognitive function	
4. Essentially normal functional activities	
5. Absence of dementia	
MCI=mild cognitive impairment.	

**Figure 3.** Petersen's MCI Diagnosis criteria. Source: Nelson & Connor (2008).

According to numerous studies, this syndrome is regarded to present a high risk of progression to dementia compared with the healthy aged population, and particularly of the Alzheimer type (Shah, Tangalos, & Petersen, 2000; Farias, Mungas, & Jagust, 2005; Ronald C Petersen & Bennett, 2005). For example, following Petersen's criteria, the conversion rate from MCI to dementia is of about 12% per year and up to 80% during approximately 6 years, while healthy controls convert at a 1–2% rate (R C Petersen, 2001; 2004). Nevertheless, some MCI patients remain clinically stable or even revert to a "normal" clinical situation over time (Larrieu et al., 2002; Ganguli, Dodge, Shen, & DeKosky, 2004; Ritchie, 2004). It is important to point out that conversion rates from MCI to dementia will be differ depending on whether studies are based in population or clinical samples, which is not surprising according to the heterogeneous nature of the MCI. Therefore, it is indispensable to make a global and rigorous assessment of the subjects in order to establish a clear diagnosis, as there are comorbid conditions, apart from neurodegenerative disorders, that could affect to the cognitive functioning, such as psychiatric status, education or hormonal changes (Gauthier et al., 2006).

There are different scales which are useful to characterize subjects along a continuum from normal aging to dementia that continue to be used nowadays by clinicians. For example, the Clinical Dementia Rating (CDR) (Morris, 1993) distinguishes from normal (CDR 0) through questionable dementia (CDR 0.5) to mild (CDR 1), moderate (CDR 2) and severe dementia (CDR 3). Individuals with CDR 0.5 would be classified as MCI. Another scale is the Global Deterioration Scale (GDS) (Reisberg, Ferris, de Leon, & Crook, 1982) which identifies seven clinical stages, from normal (GDS 1), to normal with SMC (GDS 2), mild dementia (GDS 3) and more severe stages of dementia (from GDS 4 to GDS 7). An MCI patient would have GDS 3. Finally, one of the most commonly used tests in the clinical context is the MMSE (Folstein, Folstein, & McHugh, 1975; Lobo, Ezquerra, Gómez Burgada, Sala, & Seva Díaz, 1979) which is a widely screening used method to detect cognitive impairment. It includes 30 questions to examine orientation, retention, attention, calculation abilities, language, memory and visuospatial skills. The cutoff for dementia is usually set at 24 points.

These tools are screening or severity rating scales, no diagnostic instruments. Therefore, clinical judgment and neuropsychological measures are always required to identify the presence of behavior and psychological disorders and also to explore the impairment in different cognitive domains. A neuropsychological assessment should include a variety of episodic memory test to check both immediate and delayed recall of verbal and nonverbal material, such as the Logical Memory I and II of the Wechsler Memory Scale, the Boston Naming Test and the Clock Drawing- order. Beyond memory, other cognitive domains should be examined, such as: executive functions (i.e. Trail Making Test B, Phonemic Fluency, Rule shift Cards and Indirect digit Span Test), language (i.e. Semantic Fluency and Boston Naming Test), visuospatial skills (i.e. Visual Object and Space Perception Test, Clock Drawing Test-copy), praxis (i.e. Ideomotor Praxis of Barcelona Test) and attention (i.e. Trail Making Test A and Direct digit Span Test). It is considered that MCI patient's scores on cognitive test are at least 1 standard deviation (SD) below average for their age and education matched group (Jelic et al., 1996), being the memory impairment the most common domain affected among MCI patients who afterwards progress to AD dementia (R C Petersen et al., 2001; Nelson & O'Connor, 2008; Albert et al., 2011).

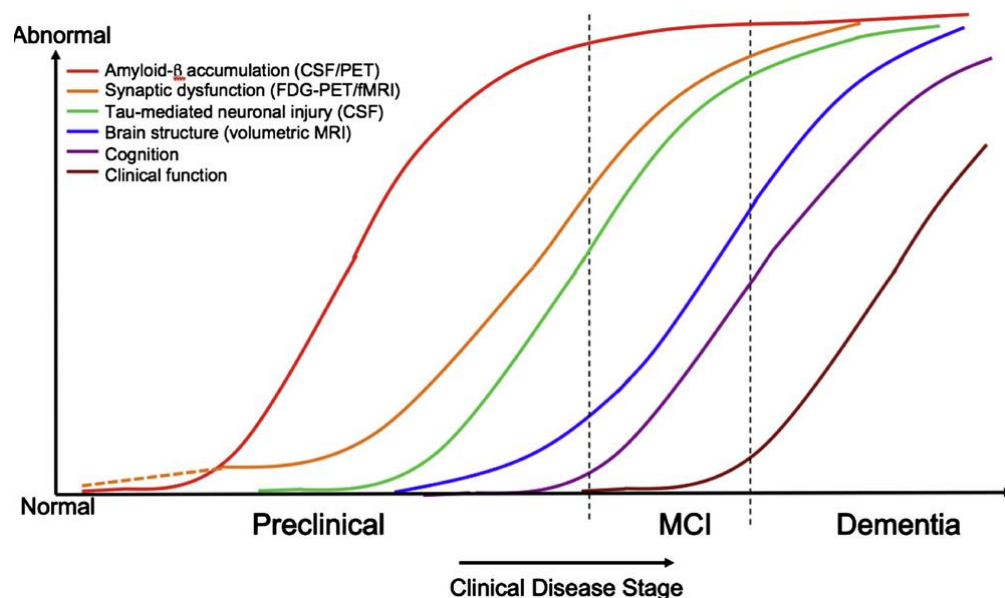
Clinical and neuropsychological information would be relevant to establish a clinical diagnosis and to delineate specific MCI subtypes. Although the initial conception of MCI was exclusively focused on deficits in episodic memory (i.e. the ability to learn new information); it is now understood that MCI may entail symptoms in other cognitive domains such as executive functions, visuospatial skills, language and attention. This new perspective classified patients

according to two orthogonal axes. In the first one, patients whose main deficit is memory are defined as “amnestic MCI” (a-MCI), while those with deficits in other cognitive functions but not in memory are classified as “non-amnestic MCI” (na- MCI). In the second axis, patients are categorized according to the number of affected cognitive domains. So, patients with only one cognitive domain affected (e.g. memory or executive function) are named “single domain” MCIs (sd- MCI), while those with at least two altered domains (e.g. memory plus executive function) are categorized as “multidomain” MCIs (md-MCI). The combination of these axes gives rise to the nowadays more broadly utilized classification of MCI subtypes: amnestic single domain MCI (a-sd-MCI), amnestic multidomain MCI (a-md-MCI), non- amnestic single domain MCI (na-sd-MCI), and non- amnestic multidomain MCI (na-md-MCI). This classification is important as MCI subtypes predict specific dementia syndromes. For example, according to Petersen’s group, the amnestic subtypes (including single and multidomain) represent a prodromal form of AD and vascular dementia (R C Petersen, 2004), whereas non-amnestic subtypes (including single and multidomain) might be at higher risk of conversion to Lewy-Body, Vascular or Fronto-temporal dementias (R C Petersen, 2004; Winblad et al., 2004). The study of the clinical subtypes of MCI is essential since they are not only associated with different etiologies but also with a more or less rapid conversion to dementia (Brodaty et al., 2012; Tabert et al., 2006).

		Cause			
		Degenerative	Vascular	Psychiatric	Medical disorders
Clinical classification	Amnestic mild cognitive impairment	Single domain Alzheimer's disease		Depression	
	Multiple domain Alzheimer's disease		Vascular dementia	Depression	
<hr/>					
Non-amnestic mild cognitive impairment	Single domain Frontotemporal dementia				
	Multiple domain Dementia with Lewy bodies		Vascular dementia		

**Figure 4.** Outline of the syndrome of mild cognitive impairment. Figure shows mild cognitive impairment with predominantly amnestic versus non-amnestic neuropsychological features, potential prodrome to neurodegenerative disorders such as Alzheimer’s disease, frontotemporal dementia, Lewy body disease, or caused by vascular cognitive impairment, psychiatric disorders such as depression or as a prodrome to other medical disorders, including metabolic and nutritional deficiencies, upper airway obstruction, and head trauma. Source: Figure adapted from Petersen & Morris (2005) by Gauthier (2006).

Besides these characteristics of the clinical syndrome (Core clinical criteria), recent proposals of MCI diagnostic (M. S. Albert et al., 2011) consider the incorporation of clinical research criteria (i.e. the use of biomarkers). They claim the utilization of a very strict terminology as the concept “MCI due to AD”, which refers to the symptomatic prodementia phase of AD. As in the case of AD, biomarkers in MCI should reflect A $\beta$  deposition (i.e. CSF and PET), total tau or phosphorylated- tau deposition (i.e. CSF) or signs of neural injury, such as brain atrophy, loss of hippocampal volume and hypometabolism or hypoperfusion measured with MRI, PET and single-photon emission computed tomography (SPECT) imaging. It is important to emphasize that the application and validation of these biomarkers is currently limited, and further studies are needed to provide an accurate diagnosis based on these criteria.



**Figure 5.** Hypothetical model of dynamic biomarkers of the AD expanded to explicate the preclinical phase: Ab as identified by cerebrospinal fluid Ab42 assay or PET amyloid imaging. Synaptic dysfunction evidenced by fluorodeoxyglucose (F18) positron emission tomography (FDG-PET) or functional magnetic resonance imaging (fMRI), with a dashed line to indicate that synaptic dysfunction may be detectable in carriers of the 34 allele of the apolipoprotein E gene before detectable Ab deposition. Neuronal injury is evidenced by cerebrospinal fluid tau or phospho-tau, brain structure is evidenced by structural magnetic resonance imaging. Biomarkers change from normal to maximally abnormal (y-axis) as a function of disease stage (x-axis). The temporal trajectory of two key indicators used to stage the disease clinically, cognitive and behavioral measures, and clinical function are also illustrated. Figure adapted from Jack et al (2010) by Sperling et al (2011).

The early detection and the prediction of cognitive decline or progression to dementia among MCI patients are still a critical issue regarding the development of interventions to prevent or delay the process of neurodegeneration (Jelic, Kivipelto, & Winblad, 2006). For this purpose, new direct assessments of neuronal damage are needed, such as the Magnetoencephalography.

### 3. MAGNETOENCEPHALOGRAPHY

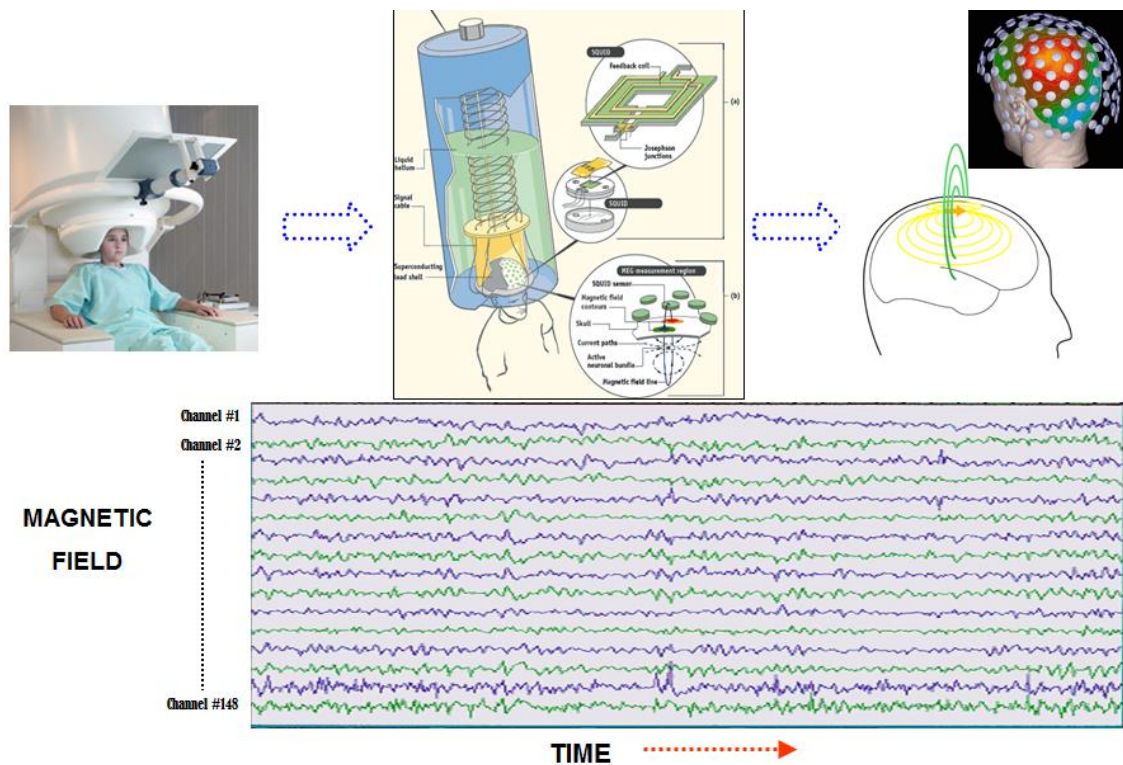
Magnetoencephalography (MEG) is a technique which emerged in the late 1960s and during the last decades its development has allowed shifting from the study of basic cognitive processes to higher cognitive activity, providing a better understanding of the brain functioning.



**Figure 6.** Elekta MEG system. Brain activity can be recorded when the subject is lying down (left) or sitting (right)

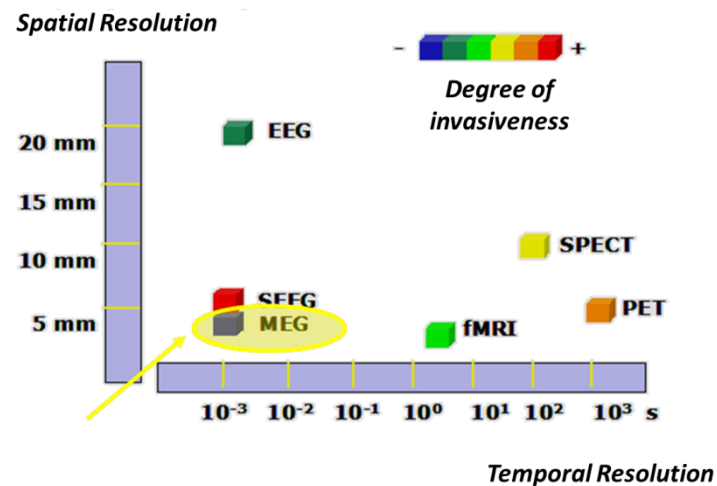
It is a noninvasive procedure which captures the weak magnetic fields of postsynaptic (intracellular) currents from a large number of the pyramidal neurons ( $10^5$ ) of the brain (Hari, 1990). These cells are mainly situated in the V layer of the cortex and postsynaptic potentials come from their apical dendrites being both inhibitory and excitatory. As electric signals generate perpendicular magnetic fields, MEG is more sensitive to capture the magnetic activity from those neurons whose dendrites are oriented tangentially to the scalp, that is, from the sulci's brain.





**Figure 7.** Process of acquisition of the MEG signal

One of the main advantages that this technique offers compared with others neuroimaging tools is its higher temporal resolution, since it allows the recording of cortical dynamics on a millisecond scale. Besides, it also provides a good spatial resolution, although it presents more difficulties to measure deeper brain activity, as the magnetic field degrades as the square of the distance. Both features are essential in the study of brain functioning since they allow not only to specify the brain structures that are involved in cognitive processes but also detail the time course of their activation.

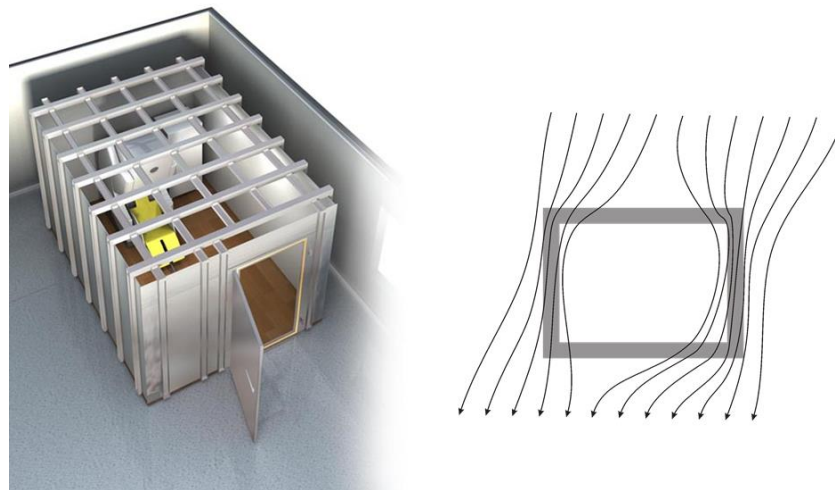


**Figure 8.** Graphic showing the relative spatial and temporal resolutions of common neuroimaging techniques (EEG: Electroencephalography, IEEG: Invasive Electroencephalography, MEG: Magnetoencephalography, MRS: Magnetic Resonance Spectroscopy, fMRI: functional MRI, SPECT: Single Photon Emission Cranial Tomography, and PET: Positron Emission Tomography). Source: Zamrini et al (2011).

Electroencephalogram (EEG) is the other noninvasive tool that provides information about brain oscillations with millisecond precision. In contrast to PET and fMRI, EEG and MEG measure the neural activation directly instead of provide indirect measures such as blood flow or metabolism, and allow repeated measurements without any risk for the subjects. However, MEG presents some advantages over the EEG as for example: 1) a better disposition for source estimation, since magnetic signals are not disturbed for the skull and other tissues (blood vessels, meninges...); 2) a better signal/noise for higher frequency bands ( $> 45\text{Hz}$ ) and 3) a better calculus of connectivity measures, as reference electrode is not needed.

The brain magnetic activity is within the order of magnitude of  $10^{-11}\text{ T}$ , equivalent to one billionth of the strength Earth's magnetic field, and the capture of this tiny signal is possible by the development Superconducting Quantum Interference Device or SQUID (Zimmerman, Thiene, & Harding, 1970), which allows the detection of the brain's magnetic activity without an electric reference (Cohen, 1972). To maintain its superconductivity capacity, the SQUID's system needs to be immersed in a Dewar filled which contains liquid helium at  $-269$  of temperature, being the mainly reason for the high cost of this technique. The MEG system also needs devices to bring the magnetic signal to the SQUID. These flux transformers could be magnetometers, which are more sensitive to measure deep magnetic fields but also external noise, and gradiometers which are more sensitive to measure superficial activity and to avoid ambient magnetic interference. Within gradiometers, planar

gradiometers presents a higher spatial resolution at the surface whereas axial gradiometers show a better depth resolution. Apart from the great sensitivity of the magnetic measurements, the external noise must be attenuated. For that purpose, the MEG system is usually located in an isolated and magnetically shielded room which is composed of layers of aluminum and  $\mu$ -metal.

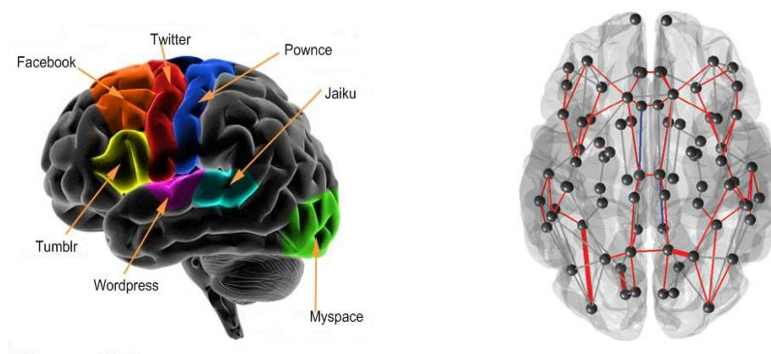


**Figure 9.** The isolated and magnetically shielded room where the MEG is located

For the MEG studies described in this Thesis a 306-channel Vectorview system (ElektaNeuromag) localized at the Center for Biomedical Technology (Madrid, Spain) was used. This MEG system comprised 102 magnetometers and 204 planar gradiometers. Only magnetometers were used for all the analysis in order to compare our results with previous ones since most of the AD and MCI studies employed the information provided by this kind of sensors.

#### 4. THE BRAIN AS AN OSCILLATORY SYSTEM

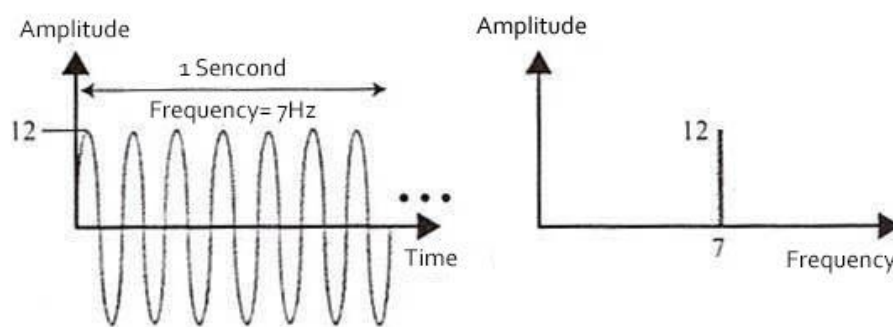
Brain function should be understood and addressed from two main perspectives: functional segregation and integration. Functional segregation refers to the specialization of local groups of neurons or brain areas (e.g. specific neurons of primary visual area are specialized to respond to vertical orientation of the stimulus), whereas the functional integration alludes to the dynamic interaction among different systems to generate brain complex brain functions (e.g. describe that stimulus). The equilibrium between segregation and integration is the responsible of an optimal brain functioning (Tononi, Sporns, & Edelman, 1994; Varela, Lachaux, Rodriguez, & Martinerie, 2001; Buzsáki, 2006; Stam & Straaten, 2012). The comprehension of the brain as a complex and dynamic entity has been possible thanks to the development of neuroimaging techniques and the analysis of the data, and the interdisciplinary approach of the Neuroscience research.



**Figure 10.** The brain understood as segregation (left) or integration (right) system. Source: Nehatiwari and Lim et al (2013)

Traditionally, human brain was described according to its anatomical structures which are highly specialized in particular cognitive functions (e.g. hippocampus and episodic memory). However, the incorporation of the idea of neuronal networks supporting cognitive functions lead to the concept of functional integration. Thus different brain regions collaborate to each other in order to support specific or multiple cognitive functions. One way to test these ideas of functional integration was by taking the advantage of an additional dimension, time, allowing a better understanding of the interdependencies that occur in the brain at the functional level. The temporal structure of the brain is organized in oscillations, which are rhythmic or repetitive small or large-scale fluctuations of the neuronal activity. The synchronous activity of oscillating neural networks allows the communication among the different brain areas and is considered to be responsible for the behavior and the cognition (e.g. perception, memory, and attention).

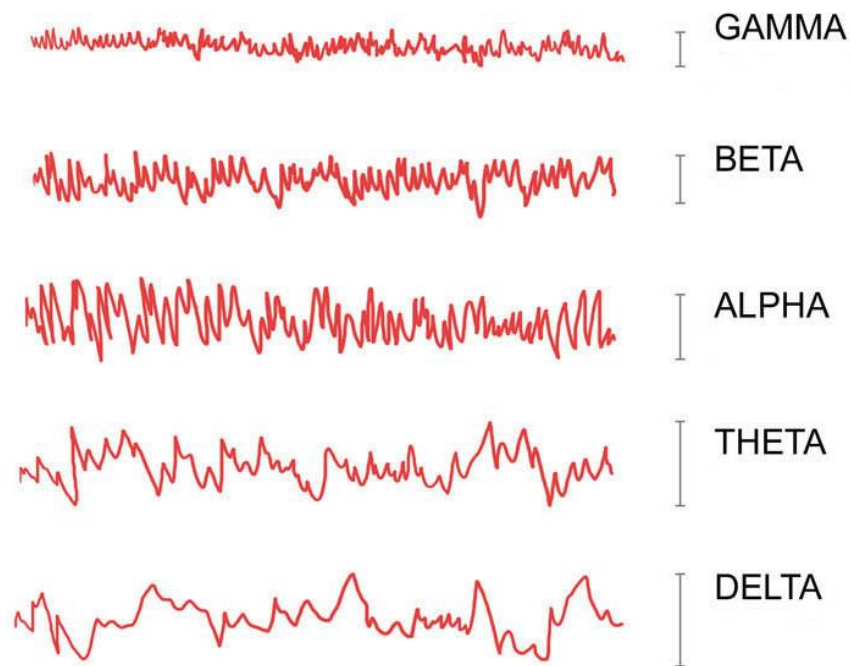
Time allows characterizing the behavior of a wave in three parameters: 1) frequency, which is the number of occurrences of a repeating event per unit of time (it is the reciprocal of the period); 2) amplitude, that is the maximum change of the wave over a period; 3) and phase, which is the relative position of the wave at a time instant. It has been described that large networks are recruited during slow activity, with oscillation periods of several seconds, whereas higher frequency oscillations are restricted to a small neuronal space, where one cycle lasts a few milliseconds (Csicsvari, Jamieson, Wise, & Buzsáki, 2003; Steriade, 2001). These findings point out the importance of studying the brain activity in temporal and spatial terms, since the frequency of neural oscillations depends on both.



**Figure 11.** Graphical representation of an analogic signal in time and frequency domains

Hans Berger described the first human electroencephalography pattern that was around 8 to 12Hz and mainly localized in occipital areas (Berger, 1929). It was called “alpha band” and is considered the dominant rhythm in the human brain. After that discovery, different rhythms were identified, being both EEG and MEG the techniques employed to measure the macroscopic oscillatory electric fields of the brain. These rhythms were arbitrarily divided in five classical frequency bands, which in ascending order are: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (>30 Hz). Thus, delta band is most prevalent in the first 2 years of life and in adults, it is normally seen during drowsiness and early slow-wave sleep (NO-REM) but if it is observed during wakefulness, it is usually a marker of brain pathology although it could increase its power in task such as in mental calculation; theta band appears in the early stages of NO-REM sleep, and in situations of hypnosis or deep meditation. It is also associated with activities that require attention and with memory processes, such as working memory, and it is present in a high proportion of children from 1 to 6 years; alpha oscillations are observable during periods of relaxation, with eyes closed but still awake and are blocked with opened eyes and is related to inhibitory process. Beta band appears with moderate levels of activation, during motor responses and increases with

cognitive effort or emotional implications of tasks, or stimuli. Finally gamma waves are associated with perception, attention and cognitive tasks. It has been described that within the same neuronal network, nearby frequency bands may coexist or compete with each other, reflecting different brain states; whereas in the same or different brain regions, oscillations of different frequencies may coincide and interact with each other (W Klimesch, 1999; Steriade, 2001).



**Figure 12.** Depiction of the five frequency bands most studied with EEG/MEG: Delta (0.5-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta (12-30 Hz) and Gamma (>30 Hz)

The assumption of non-stationary of the oscillations involved in brain functioning is crucial to select the methodology to analyze them, and therefore the spectral characterization can be done taking in to account the time domain. Fourier transform is a mathematical transformation which provides the spectral power through the decomposition of a stationary function that exists throughout the entire duration of the signal, in a sum of sinusoidal components of different frequencies. As this classical analysis does not inform about the point of time of appearance of these spectral components, the time-frequency analysis is suitable for obtaining such information at a well-defined time (Le Van Quyen & Bragin, 2007). Both types of analysis are useful to characterize the activity of neurons or local brain regions, but to explore how brain networks communicate with each other, the study of synchronization is required. To evaluate the synchronization between brain regions, the concept of functional connectivity has emerged. It refers to linear or nonlinear statistical interdependencies between time series of physiological signals recorded from different brain areas, providing

information about functional interactions between the underlying brain regions (Friston, 2001).

Spectral power and functional analysis supply essential information about how brain works in healthy and pathological states. However, it should be noted that band power and its interpretation in terms of (local) levels of neuronal synchronization is not as direct and straightforward as we could think about (Daffertshofer & van Wijk, 2011).

In order to explore the data from different methodological perspectives, this Thesis begins with a more classical approach (power in sensor space) to end with the newest analysis in MEG research (functional connectivity in source space).

## 5. RESTING STATE IN AD AND MCI PATIENTS

### Slowing of background activity

Hans Berger first observed the correlation between the cognitive decline in AD patients and the changes detected in EEG recordings (Berger, 1931; 1932). Most EEG (Babiloni et al., 2004; Dauwels et al., 2011; Huang et al., 2000; Jeong, 2004) and MEG studies (Berendse, Verbunt, Scheltens, van Dijk, & Jonkman, 2000; Fernández et al., 2002, 2003; Osipova, Ahveninen, Jensen, Ylikoski, & Pekkonen, 2005) have found the so- called global “slowing” in AD. It is characterized by an increase in power in low-frequency range that includes delta and theta bands, and by a power decrease in higher frequency range which includes alpha and beta bands. Additionally, topographical changes occurs in the distribution of the alpha and beta rhythms, which relocate towards anterior areas as the disease progresses (Babiloni et al., 2004; Claus et al., 1998; Dierks, Ihl, Frölich, & Maurer, 1993); and also in low frequency bands, which are more prominent in the left temporal area (Rice et al., 1990; Buchan et al., 1997; Rodriguez, Copello, Vitali, Perego, & Nobili, 1999; Fernandez et al., 2002). The degree of the brain abnormality measured by EEG/MEG has been associated with APOE genotype, cognitive impairment, hippocampal atrophy and frontal white matter loss (Lehtovirta et al., 2000; Fernández et al., 2003; Kwak, 2006; Babiloni et al., 2006c; Gianotti et al., 2007). It is well establish that in the early stages of the disease there is an increase in theta band accompanied by a decrease in beta activity, followed by a decreased in the alpha power. As delta activity increases as the disease progress, patients with mild dementia show an increase in theta and a decrease in beta bands whereas patients with moderate severe dementia present a decrease in alpha and a rise in delta power (Coben, Danziger, & Storandt, 1985; Hier et al., 1991).

Many EEG/MEG studies show that MCI patients tend to present intermediate values between healthy controls and AD patients in their power spectrum. The most stable pattern of EEG activity in MCI patients is defined by an increase of theta and to a lesser extent of delta, accompanied by a decrease of alpha and beta power (Prichep et al., 1994, 2006; Babiloni et al., 2010; Dauwels et al., 2011; Fernández, Hornero, et al., 2006b; D. V Moretti et al., 2007;). In addition, some studies have also correlated these changes with APOE genotype, frontal white matter and hippocampal volumes (Babiloni, Benussi, et al., 2006a; Babiloni, Binetti, et al., 2006a; Babiloni, Frisoni, et al., 2006c; Babiloni et al., 2009, 2010). Of note, the investigation of differential neurophysiological patterns in MCI subtypes is scarce. This issue was indirectly addressed by Babiloni et al. (2009) within the background of a research on the relationship between hippocampal volumes and alpha rhythms. The authors assessed a potential influence



of the clinical subtype (i.e. a-MCI vs. na-MCI) and found no significant differences. In a subsequent study (Babiloni et al., 2010), the issue was explicitly investigated by comparing EEG rhythms in a-MCIs, na-MCIs, and aged subjects with SMC. Results showed increased occipital theta and reduced alpha activity in a-MCI, as compared to na-MCI. No distinctions were made in terms a single or multidomain affectation.

It should be noted that there are also EEG/MEG changes during healthy elderly. The main modification occurs in alpha band. For example, Chiang, et al. (2011) found that frequency increases from childhood to young adulthood and slowly begins to decrease with age; Dustman, et al. (1993) exhibited that apart from this a slowing of alpha frequency, there was an increase in slow wave activity (delta and theta) and a trend to an increase in beta activity; and finally Fernández et al. (2006a) described a frequency decrement of 0.17Hz per year in this group. All this findings suggest that a slowing-down in brain activity is a normal feature of healthy aging, however it is important to point out that the EEG/MEG pattern observed in MCI patients is quantitatively different from that observed in the control subjects.

### **Changes in functional connectivity**

It has been suggested that AD can be considered as a “disconnection syndrome” (Delbeuck, Van der Linden, & Collette, 2003). From a structural point of view, anatomical links in AD patients are disrupted and this has been associated with a neurofibrillary pathology, hypometabolism and brain atrophy (Braak & Braak, 1991; Frisch et al., 2013; C L Grady, Furey, Pietrini, Horwitz, & Rapoport, 2001; Salmon, Lekeu, Bastin, Garraux, & Collette, 2008). And at a functional level, a decrease in cortico-cortical interactions has been shown in many EEG/MEG studies. Berendse et al. (2000) described a general decrease of coherence in all frequency bands; although most functional connectivity studies have found a lower synchronization mainly in alpha and beta bands in AD patients compared to healthy controls (Locatelli, Cursi, Liberati, Franceschi, & Comi, 1998; C.J. Stam & van Dijk, 2002; C J Stam, van der Made, Pijnenburg, & Scheltens, 2003; Babiloni et al., 2004; see Jeong, 2004 for a review; Koenig et al., 2005). Additionally, the specific changes of long and short distance interactions found in this population has been correlated with disease severity as expressed by a lower MMSE score (C J Stam et al., 2003; 2006), suggesting that the disruption of the anatomical/ functional connections is potentially responsible of the cognitive alterations that characterized this pathology. Finally, as the disease progresses, some studies have found an increase in connectivity in slow frequency bands (delta and theta), possibly as a result of the cholinergic

cortical deafferentation from subcortical structures (Locatelli et al., 1998; Pijnenburg et al., 2004; C J Stam et al., 2006).

Since MCI patients present neuropathologically features that could be considered intermediate between normal aging and early AD (He et al., 2009; Ronald C Petersen et al., 2006; Zhang et al., 2012), the concept of “disconnection syndrome” might also be applied in this group. Many studies in MCI have found a decrease in synchronization, which would support this idea. Moretti et al. (2008) reported a decline in intrahemispheric coherence in fronto-parietal regions and an increase in interhemispheric coherence on frontal and temporal areas in the MCI group. Gómez, et al. (2009) and Koenig et al. (2005) found a decreased synchronization essentially in the beta band in MCI subjects when compared to elderly controls. Meanwhile Babiloni, Ferri, et al. (2006d) found significant differences in synchronization between MCI subjects and normal elderly subjects in delta and alpha bands at fronto- parietal regions and it was related to MMSE scores. However, other resting state studies have failed to find functional connectivity differences between MCI and healthy controls (Stam et al. 2003; Tao and Tian 2005; Jiang 2005; Zheng et al. 2007). By contrast, most cognitive studies have described clear differences between MCI patients and healthy people in functional connectivity during the execution of a cognitive task (Pijnenburg et al. 2004; Jiang 2005; Zheng et al. 2007; Bajo et al. 2010). All these results suggest that is essential studying both resting and cognitive states in order to distinguish them from controls, since the heterogeneity that characterizes the MCI patients makes that often the results are not as consistent one might have expected.

## 6. INTERNALLY DIRECTED COGNITIVE STATE (IDICS)

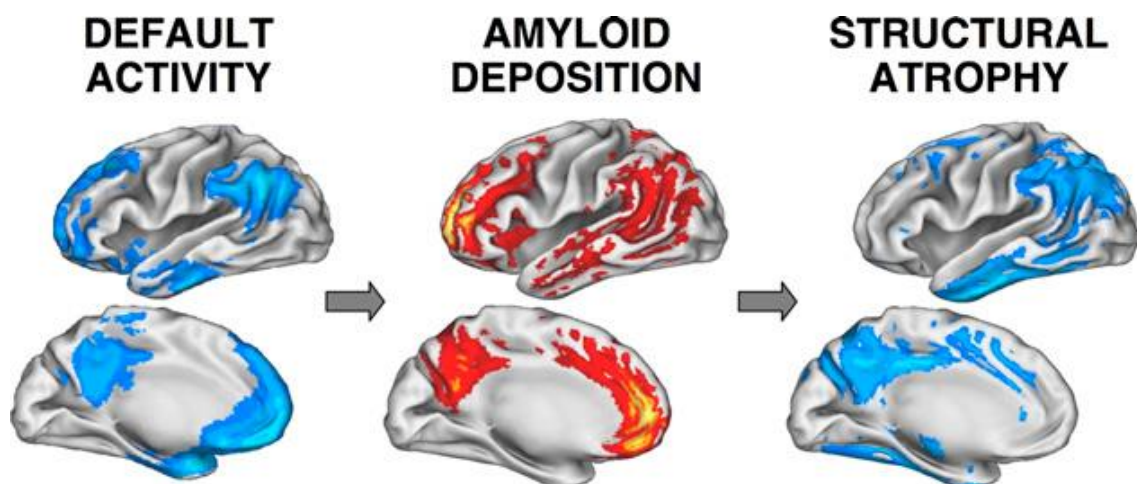
The investigation of resting state activity has become an extremely prominent topic in neurocognitive research. But, are our minds really do nothing during resting state? Actually, our brains are usually engaged in spontaneous cognition or “mind-wandering” during these periods of rest or even during undirected passive tasks. Both concepts are referred to thoughts commonly related to autobiographical memories, future planning, social interactions and personal problem solving. All these thoughts are essential for humans since they provide us an adaptive value and a continuous feeling of “self” (Andrews-Hanna, Reidler, Huang, & Buckner, 2010; 2011). In fact, spontaneous thinking is so important for us that the technique of so-called experience sampling estimate that humans spend between 30% and 50% of daily life involved in thoughts unrelated to the immediate task at hand (Killingsworth & Gilbert, 2010). One plausible hypothesis about the differences frequently found in neuroscience studies during resting state could be due to individual and cultural differences in the frequency and contents of mind wandering (Callard, Smallwood, & Margulies, 2012; Maillet & Rajah, 2013; Song & Wang, 2012).

Resting state brain activity has been related with a set of brain areas which are more activated during passive baseline conditions, and deactivated during external and goal directed cognitive activity, as a kind of task-negative baseline network. This group of regions has been named the “Default mode network” (DMN) (Raichle et al., 2001) and mainly includes the posterior cingulate cortex/ precuneus, dorsal and ventral medial prefrontal cortex, lateral (principally inferior) parietal cortices and medial temporal lobes (Buckner, Andrews-Hanna, & Schacter, 2008; Fox et al., 2005; Mevel, Chételat, Eustache, & Desgranges, 2011). These regions seem to be the most sensitive to the neurodegenerative disorders, such as AD (Mevel et al., 2011).

The DMN activity measured by fMRI is characterized by coherent low frequency synchronized oscillations of BOLD signal time series ( $<0.1$  Hz) in large- scale functional brain networks (Broyd et al., 2009) that persists during simple sensory tasks, such as visual fixation (Raichle et al., 2001), during the early stages of sleep (Horovitz et al., 2008) and to a lesser extent, under conscious sedation (Greicius et al., 2008). It is considered that this network may be involved in exploratory monitoring of the external environment when focused attention is relaxed or diffused (e.g. in passive conditions), and other times its activity is related to mind wandering (Andrews-Hanna, 2012; Buckner et al., 2008; Sonuga-Barke & Castellanos, 2007).

The DMN plays an opposite role than the task- positive network, whose regions routinely exhibiting task-positive responses (Fox et al., 2005). Both networks maintain an anti-correlated relationship that allows the adaptation of the brain's resources to the external or internal demands. For this reason, the intensity of the deactivations of the DMN depends on the load or the cognitive demands of the external task (Persson, Lustig, Nelson, & Reuter-Lorenz, 2007; Sambataro et al., 2010).

Although the group of brain regions of the DMN is consistent across the lifespan, the magnitude of its activation as well as the functional correlations among its areas seem to be reduced in aging (Andrews-hanna et al., 2009; Cheryl L Grady, Springer, Hongwanishkul, McIntosh, & Winocur, 2006; Koch et al., 2010). These changes suggest that old people show a reduction in the ability to suppress the DMN activity when higher processes are needed, implying an obstacle in switching from a "default mode" to task- related mode of brain function, and consequently their performance on cognitive tasks is worse. Moreover it should be note that these disturbances are greater in MCI and AD since DMN structures are vulnerable to atrophy, deposition of amyloid-plaques and present a decrease glucose metabolism (Minoshima et al., 1997; Scahill, Schott, Stevens, Rossor, & Fox, 2002; Buckner et al., 2005). Therefore the study of the DMN is particularly relevant in aging and dementia (for a review see Hafkemeijer et al. 2012).



**Figure 13.** The default mode network. The left side shows cerebral regions with coherent default network activity under resting conditions in young adults. These resemble areas of both increased amyloid plaque deposition assessed by molecular imaging modalities such as PET (middle) and of cortical atrophy measured by morphological MR-imaging (right). The similarity might be explained by steady increased baseline activity in default networks leading to an increased pathology with subsequent neurodegeneration. Figure adapted from Buckner et al (2005) by Wermke (2008).

Based on the previous ideas I wondered, what would it happen in the DMN if we employed an experimental paradigm which combine the resting state and a cognitive task?

Most studies in cognitive functioning, and particularly in dementia, have been based on external stimuli; whereas those that have explored spontaneous or goal-directed internal mentation have focused on mind wandering (Andrews-Hanna et al. 2010; 2012). However, little is known about brain activity and more specifically about the DMN during internally guided cognition, despite knowing that people also spend their time engaged in solving mathematical problems, hypothesizing or checking their knowledge. For this reason the concept of “Internally Directed Cognitive State” (IDICS) was proposed. But, what kind of cognitive task could be the most appropriate to apply, in an internal way, in healthy and pathological aging? One in which patients show difficulties in its execution and in which internal processing and self-monitoring would be required to perform it, in order to explore how brain activity changes with the presence of a pathology. For this reason, we proposed an internal mental calculation task in Study II.

Among other cognitive deficits, mental calculation abilities are commonly impaired early in the course of AD (Parlato et al., 1992; Rémy et al., 2004). This impairment correlates to the degree of dementia (Marterer, Danielczyk, Simanyi, & Fischer, 1996; R. C. Martin et al., 2003) and with glucose hypometabolism in the left inferior parietal lobule, left inferior temporal gyrus and prefrontal regions (Hirono et al., 1998; Rémy et al., 2004). Simpler arithmetic tasks-such as addition or subtraction are related to language and long-term semantic memory (Dehaene, Molko, Cohen, & Wilson, 2004) and require executive processes such as working memory (WM) (Hitch, 1978). Brain structures such as prefrontal and superior parietal areas are involved in calculation tasks and are affected in MCI (Singh et al., 2006), apart from the well-known atrophy of the hippocampus and other medial temporal lobe regions (C R Jack et al., 2000). However, few studies have focused on calculation in MCI, and they indicate that this group had difficulties in simple calculation tasks. Zamarian et al., 2007a; 2007b) demonstrated that simple calculation in MCI was well preserved, but they presented problems when inhibition processes were required. Otherwise, Li et al. (2010) found that MCI patients performed worse than healthy controls, during the execution of a simple mental calculation task, and showed abnormalities on N170 and P2 ERP components.

It should be pointed that IDICS is a cognitive state that simulate resting state but in which a cognitive task is carried out. Therefore, it characterizes for not depending on the external environment and having a purely cognitive content (formal content), not self-referential. The main objective of IDICS is focused on the study of the internal processing

involved during the execution of cognitive activities, and therefore, any cognitive task whose execution requires the creation of cognitive internal representations and is internally directed could be addressed from this new approach, such as mental calculation.

Thus, IDICS apart from allowing the exploration of the human mind's nature when performing cognitive tasks in the absence of external stimuli, offers a high flexibility since it can be applied to different populations and be based on diverse cognitive tasks (executive function, memory...); it also may provide information about the differences between internal and external processing and also about the ability to concentrate and vulnerability to distraction, may contribute to generate a new homogenous scenario to make inter-and intra-individual comparisons, and finally it may supply essential information about the behavior of the DMN in comparison with introspective mentation (Andrews-Hanna, 2012) in a large number of neurological and psychiatric disorders in which it is altered (Broyd et al., 2009; Buckner et al., 2008).

## 6. PROGRESSION FROM MCI TO AD

There is a broad consensus in considering the MCI as a precursor of AD, since many longitudinal studies have found a high rate of progression from this state to AD. In general, the conversion rate of MCI to dementia is around 10-15% per year (R C Petersen et al., 2001a), although this value may vary due to the MCI criteria applied, the type of sample used (community or clinic-based), as well as to the length of the follow-up period considered (Jelic et al., 2000). The early identification of those MCI patients who will convert to AD is essential to initiate an adequate administration of pharmacologic or non-pharmacologic interventions to slow down the devastating effects of this neurodegenerative disorder.

From a neuropsychological point of view, the performance in episodic memory tests, such as new learning, has been proposed as a predictor of conversion from MCI to AD (Albert M, Blacker D, Moss MB, Tanzi R, 2007; Perri, Serra, Carlesimo, & Caltagirone, 2007; Lekeu, 2010). Besides, some studies have suggested that also the alteration in other cognitive domains, especially in executive functions, may have a predictive power of progression (Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST, 2000; Rozzini L, Vicini Chilovi B, Bertoletti E, Conti M, Delrio I, Trabucchi M, 2008; Brandt et al., 2010; Lekeu et al., 2010; Chapman et al., 2012). These results could be explained on one hand, due to the MCI criteria selected, as some studies differentiate among MCI subtypes while others not; and on the other hand, because the cognitive exploration is not enough detailed as it should be. Thereby, there are studies that have found that a-md-MCI sample constituted the high-risk group to progress to AD, compared to a-sd-MCI and non-amnesic subtypes (Ronald C Petersen & Bennett, 2005; Tabert et al., 2006; Brodaty et al., 2012), and others have described that even pure amnesic MCI patients presented impairments in executive function (Griffith et al., 2003; Johns et al., 2012; Kramer et al., 2006). These findings highlight the importance of making a rigorous neuropsychological assessment with the aim to establish an appropriate diagnosis and prognosis in this population (Tatsuoka et al., 2013).

Apart from this clinical approach, current studies are focusing on both structural and molecular biomarkers in order to prospectively identify those patients who will subsequently manifest AD. MRI studies of conversion from MCI to AD have already been associated with hippocampal and entorhinal volume loss (deToledo-Morrell et al., 2004; Devanand et al., 2007), although atrophy in other brain regions; such as parietal cortex, ventricles or even the whole brain; have also been described (C. Jack, Shiung, & Weigand, 2005; Karas et al., 2008). Besides, it has been found that assessment of cerebral glucose metabolism with FDG- PET may also contribute in

predicting the evolution of MCI patients, since most of them showed that MCI converters present regional hypometabolism in the temporal, parietal and/or precuneus cortices compared with non-converters (Drzezga et al., 2003; Hatashita & Yamasaki, 2013). Moreover, recent investigations about B-amyloid (AB) and tau proteins consider these two parameters essential in predicting the progression to dementia, since they are respectively the reflection of the amyloid plaques deposition and neurofibrillary tangles that occurs in the AD patient's brain. Most of the PIB-PET studies, show that PIB-positive MCI patients are significantly more likely to convert to Alzheimer's dementia than PIB-negative (Forsberg et al., 2008; Grimmer et al., 2013). These results are in agreement with those that have found decreased levels of AB in CSF in MCI converters compared with MCI- stable, as both measured are inversely correlated (Riemenschneider et al., 2002; Hampel et al., 2004). For its part, an increase in tau protein levels in CSF in progressive MCIs have been reported in several studies (Riemenschneider et al., 2002; Lanari & Parnetti, 2009), being its predictive power even higher than in the case of AB (Hampel et al., 2004)

Nowadays, the research in dementia is focusing on the combination of different biomarkers and clinical information with the purpose of achieving higher sensitivity and specificity values for predicting progression to AD in patients with MCI (Hansson et al., 2006; Ewers et al., 2012; Richard, Schmand, Eikelenboom, & Van Gool, 2013). It should be pointed that the predictive power of some of these methods is very heterogeneous and those with high values of specificity and sensibility are often limited by their high degree of invasiveness, reducing the likelihood of a daily clinical usage. Additionally, these measures do not provide a direct assessment of the neuronal functioning and as a consequence to the neuronal network impairment. Thus, it seems necessary a non-invasive biomarker able to evaluate brain functioning at the neuronal level, such as EEG and MEG, which are two non-invasive techniques able to measure the neuronal activity and its representative functional networks in the time-frequency domain.

There is a growing body of evidence showing that the analysis of EEG patterns may provide additional information to characterized brain activity of MCI patients who become AD. Resting state EEG longitudinal studies have found that the main markers of progression to AD were a decrease in alpha activity and an increased theta power (Jelic et al., 2000). Meanwhile, Huang et al. (2000) evidenced that the best predictor of future conversion to AD within a MCI sample was the shift of alpha activity towards anterior brain. Afterwards, Rossini et al. (2006) found that MCI converters exhibit higher power values in delta, theta and alpha1 bands, mainly over temporal and parietal areas, and also changes in fronto- parietal midline



coherence values. More recently Moretti et al. (2011) observed that those MCI who convert to AD presented an increase of  $\alpha_3/\alpha_2$  relative power ratio, being this ratio further related to hippocampal atrophy (Moretti et al., 2007), and Poil et al. (2013) found that multiple EEG biomarkers were mainly related to activity in the beta-frequency range which was higher in the MCI converters than in the MCI non-converters. Otherwise, there are only few studies which have employed cognitive tasks to explore the differences between progressive MCI and stable MCI subjects, such as those carried out by Missonnier et al. (2006; 2007) who described that those MCIs who converted to AD showed a distinct EEG patterns during n- back WM tasks than those MCIs who remained stable over time.

The necessary use of a reference channel in EEG studies may introduce spurious synchronicity between signals, and therefore the MEG seems to be an optimal alternative. Surprisingly there are very few MEG studies about the MCI progression to AD. For example, a resting state MEG study from Fernández et al. (2006c) found that an increase in delta activity in left parietal areas was a good marker of conversion from MCI to AD within 2 years. In 2011 Maestú et al. observed that during the performance of a short- term memory task, progressive MCI patients showed higher activity in ventral and dorsal pathways than stable MCI subjects. Afterwards, and under the same memory paradigm, Bajo et al. (2012) described that MCI converters exhibited higher values of synchronization over parieto- occipital regions in alpha and beta bands than MCI non- converters. Prospective MEG studies, such as the evolution of MCI to AD, are needed to start considering this technique as a new non-invasive biomarker as those used nowadays.

## **STUDY I**

### **MEG spectral analysis in subtypes of Mild Cognitive Impairment**

## **1. AIMS AND HYPOTHESIS**

The main goal of this first study was to explore the brain magnetic patterns of the normal aging and the MCI, and more specifically, of its two amnesic clinical subtypes. To this end, we conducted a MEG study where an exhaustive spectral analysis was carried out in a-sd-MCIs, a-md-MCIs, and healthy aged controls. In addition, we further explored the cognitive status of the participants and their hippocampal volumes in order to assess the relationship among spectral activity, neuropsychological performance and structural atrophy information.

We hypothesize that a-md-MCI patients will exhibit a spectral pattern more proximate to the typical AD-profile; including increased power within the delta and theta frequency ranges, and reduced activity in the high-frequency range as compared with a-sd-MCIs and healthy controls. Meanwhile, a-sd-MCIs will exhibit a spectral activity in between a-md-MCIs and controls. Besides, these neurophysiological findings will be related to changes at structural and cognitive level, which will allow us to characterize the two amnesic clinical subtypes of MCI.

## 2. METHODS

### 2.1 Subjects

MEG signals were obtained from 105 subjects older than 65 years of age, classified in three groups: 36 healthy controls, 33 a-sd-MCI patients, and 36 a-md-MCI patients. All of them were right handed (Oldfield, 1971), and native Spanish speakers. No significant differences were found in education, gender, or age among groups (see Table 1). MCI patients were recruited from the Geriatrics and Neurology Units of the “Hospital Universitario San Carlos” and the “Memory Decline Prevention Center”, both in Madrid, Spain. Healthy volunteers were recruited from the “Seniors Center of Chamartín District”, Madrid.

**Table 1.** Mean values ( $\pm$  standard deviation) of the demographic and clinical characteristics of a-sd-MCI, a-md-MCI and Controls. M = males, F= females, MMSE = Mini mental state examination score. An ANOVA test was used for the comparisons.

	Control (n=36)	a-sd-MCI (n=33)	a-md-MCI (n=36)	P value
<b>Age (years)</b>	72.36 $\pm$ 4.75	74.15 $\pm$ 6.07	73.94 $\pm$ 3.70	p= 0.22
<b>Gender (M/F)</b>	11/25	13/20	13/23	p= 0.12
<b>MMSE score</b>	29.14 $\pm$ 0.96	27.63 $\pm$ 2.47	25.65 $\pm$ 2.66	p < 0.01
<b>Education (years)</b>	4.39 $\pm$ 1.23	4.27 $\pm$ 1.31	3.78 $\pm$ 1.24	p= 0.48
<b>LH_ICV</b>	0.002610 $\pm$ 0.0003583	0.002148 $\pm$ 0.0004235	0.002062 $\pm$ 0.0005280	p < 0.0001
<b>RH_ICV</b>	0.002608 $\pm$ 0.0002964	0.002133 $\pm$ 0.0005079	0.002082 $\pm$ 0.0003512	p < 0.0001

## 2.2 Diagnostic criteria

All participants were screened by means of a variety of standardized diagnostic instruments that included: the Spanish version of the MMSE (Lobo, Ezquerra, Gómez Burgada, Sala, & Seva Díaz, 1979), the Global Deterioration Scale (GDS; Reisberg, Ferris, de Leon, & Crook, 1982), the Functional assessment questionnaire (FAQ; Pfeffer, Kurosaki, Harrah, Chance, & Filos, 1982), the Geriatric Depression Scale (GDS; Yesavage et al., 1982), the Hachinski Ischemic Score (HIS; Rosen, Terry, Fuld, Katzman, & Peck, 1980), the questionnaire for Instrumental Activities of Daily Living (IADL; Lawton & Brody, 1969), and the Functional Assessment Staging (GDS/FAST; Auer & Reisberg, 1997).

MCI diagnosis was established according to the Petersen (2004) and Grundman et al.'s (2004) criteria. Thus, MCI patients should fulfill the following requirements: (1) memory complaint, corroborated by an informant; (2) abnormal memory function, documented by delayed recall of one paragraph from the Logical Memory II subtest of the *Wechsler Memory Scale-Revised* (cutoff scores  $\leq 16$  for  $\geq 16$  years of education;  $\leq 8$  for  $\geq 8$ –15 years of education); (3) normal general cognitive function, as determined by a Mini-Mental State Examination (MMSE) score greater than or equal to 24; (4) total absence or minimal impairment in activities of daily living (ADLs) revealed by the Lawton scale, as determined by a clinical interview with the patient and informant; and (5) not demented according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria as judged by an experienced clinician (G. McKhann et al., 1984). MCIs did not fulfill the diagnostic criteria for dementia (i.e. all were classified at the stage 3 of the GDS) and performed at least 1 SD below average for their age and education on neuropsychological tests representing one or more areas of cognition (Jelic et al., 1996). Patients and controls were free of significant medical, neurologic and/or psychiatric diseases (other than MCI). These inclusion criteria included the absence of significant cerebral-vascular disease (i.e. modified Hachinski score  $\leq 4$ ) or depressive symptomatology (i.e. Yesavage's Depression Scale scores  $> 9$ ). Participants were not using drugs which could affect MEG activity (including cholinesterase inhibitors).

Patients and controls received an exhaustive neuropsychological assessment in order to establish their performance level in multiple cognitive domains. The assessment included: Clock Drawing Test (Agrell & Dehlin, 1998), Direct and Inverse Digit Span Tests (Wechsler Memory Scale Revised, WMS-III; Wechsler, 1987), Immediate and Delayed Recall (WMS-III; Wechsler 1987), Phonemic and Semantic Fluency (Controlled Oral Word Association Test,

COWAT, Benton & Hamsher, 1989), Ideomotor Praxis of Barcelona Test (Peña-Casanova, 1990), Rule shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS; Norris & Tate, 2000), Visual Object and Space Perception Test (VOSP; Warrington & James, 1991), Boston Naming Test (BNT; Kaplan, Goodglass, & Weintraub, 1983) and Trail Making Test A and B (TMTA and TMTB; Reitan, 1958). The TMT subtest A and B offer two different scores. The first one (i.e. TMT “accuracy”) denotes the number of correct responses. The second score (i.e. TMT “time”) denotes the time subjects need to complete the task, with a limit of 200 seconds in TMTA and 400 seconds in TMTB.

According to their clinical and neuropsychological profile, patients were further divided in two groups: (1) the a-md-MCI group where patients showed a memory deficit accompanied by various degrees of impairment in cognitive domains such as language, executive function and/or visuospatial skills; and (2) the a-sd-MCI group where patients exhibited isolated memory impairment (Petersen 2004).

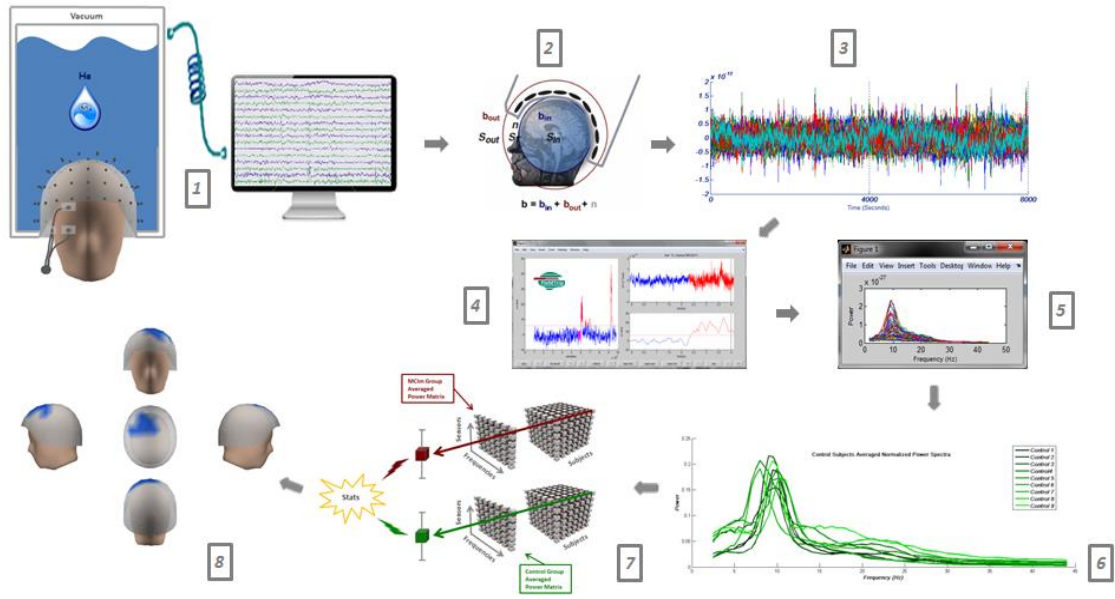
Prior to the MEG recording, all subjects signed an informed consent that explained the technical and ethical considerations of the investigation. The study was approved by the local Ethics Comitee.

### *2.3 MEG recordings*

MEGs were acquired (Figure1, step 1) with a 306-channel Vectorview system (ElektaNeuromag) which combines two orthogonal, planar gradiometers, and one magnetometer. Only MEG signals derived from magnetometers (i.e. 102 channels) were submitted for further analyses. The MEG system was placed in a magnetically shielded room (VacuumSchmelze GmbH, Hanua, Germany) at the “Laboratorio UPM-UCM de Neurociencia Cognitiva y Computacional” (Madrid, Spain). Subjects were in an awake but resting state with their eyes closed and under vigilance control during the recording. They were asked to avoid making movements. For each subject, three minutes of MEG signal were acquired at a sampling frequency of 1000 Hz (online bandpass filtering at 0.1-330Hz).

The head movement was controlled by means of a head-position indicator (HPI) with coils attached to the scalp. HPI coils position and subject headshape were defined using a 3D digitizer (FastrakPolhemus) referenced to three anatomical (fiducial) locations: the nasion and the left and right preauricular points. Blinks were monitored by two bipolar electrodes attached above and below the left eye and one electro attached to the lower cheek (ground).

Recordings were offline filtered (Figure1, step 2) and corrected for head movements with a temporal signal space separation with movement compensation (tSSS-mc) (Taulu & Kajola, 2005; Maxfilter 2.2 software); correlation threshold = 0.9, time window= 10 seconds, and notch filtered (Butterworth filter order 4 at 50 Hz and 100Hz). Continuously recorded resting state data were segmented in 4-seconds length trials. Trials with EOG, muscle and jump artifacts were rejected by means of Fieldtrip package (Oostenveld, Fries, Maris, & Schoffelen, 2011) (Figure1, step 3).



**Figure 1.** Workflow: resting state MEG recordings (1), Elekta software spatial filtering (2), signal segmentation (3), Fieldtrip artifact processing (4), visual trial selection through power spectra (5), power spectra averaging and normalization (6), statistics (7) and results (8).

## 2.4 MRI and hippocampal volumes

For each subject, a high resolution T1 weighted magnetic resonance was acquired at “Hospital Universitario San Carlos” (Madrid) by using a General Electric 1.5 Tesla magnetic resonance (MR) scanner, with a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). To segment the subject’s T1-weighted volume into different regions, Freesurfer software (version 5.1.0) and its specialized tool for automated subcortical segmentation (Fischl et al., 2002) were used. Afterwards, hippocampal volumes were normalized with the overall intracranial volume (ICV) to account for differences in head

volume over subjects. Two variables were submitted for statistical analysis: the normalized left hippocampal volumes (LH\_ICV), and the normalized right hippocampal volumes (RH\_ICV).

### *2.5 Power spectra and statistical Analysis*

MEG power spectra were computed through Fieldtrip package for all trials which successfully passed the automatic artifact rejection. A frequency-of-interest range of 0.5 Hz steps from 1 to 30 Hz was employed. In order to obtain the average frequency-content of each trial we applied a multitaper method (mtmfft) with discrete prolate spheroidal sequences (dpss) as windowing function and 1 Hz smoothing. Each trial was visually inspected by an experienced technician blinded to the subjects' diagnosis by collapsing the power spectrum of all channels (Figure 1, step 4). Those channels with an aberrant power spectra profile were dismissed. Finally, only MEG recordings with at least 15 survival trials (one minute of brain activity) were submitted for further analyses. The number of survival trials did not differ significantly among groups. Survival trials were averaged across subjects obtaining for each group a 102 channels x 52 frequency steps x "n" subjects matrix (Figure1, step 6). For each channel relative power was calculated as a ratio between power in each 0.5 Hz frequency step and the total power across the 1-30Hz spectrum, and was expressed as a percentage (Jelic et al., 2000).

Similarly to previous works (see Fernandez & Hornero, 2006b) we did not use pre-established and conventional frequency bands to analyse power differences among groups. This approach might overcome one of the key problems that emerge when different studies are to be compared: the variability in terms of classification criteria for classical EEG bands. This variability affects low-frequencies to a lesser extent; however the limits between conventional bands in the high-frequencies range are difficult to define. Thereby, in order to accomplish a data-driven comparison among groups, we followed a method adapted from Maris & Oostenveld (2007). First, a series of exploratory ANOVA tests were calculated (Figure 1, step 7) for relative power values in each 0.5 Hz frequency step and sensor. Those comparisons which were found to show significant differences ( $p < 0.05$ ) were further inspected by means of pairwise t-tests. In order to perform such analyses, a series of clusters were built according to a criteria of spatial and frequency adjacency. Thus, each cluster must contain at least 5 contiguous and significant sensors, and the difference between pairs of groups must remain significant during at least a 2Hz-interval.

Relative power on each cluster of sensors was averaged and submitted to the t-test analyses. To control the family-wise error due to multiple comparisons, the test distribution was derived



from a permutation test (Ernst, 2004). This was accomplished by randomly dividing the participants into two sets, matching the numbers in the original groups. The two-sample t-test was then carried out in these two new groups. This procedure was repeated 5000 times and the p-value from each test was retained in order to obtain a p-value distribution. We then identified the 5th percentile of each distribution, and only p-values below that threshold were accepted. This strategy was performed for the contrasts a-sd-MCI vs. control, a-md-MCI vs. control, and a-sd-MCI vs. a-md-MCI. Importantly, the criteria utilized to build each cluster (see above) determines the way in which spectral differences between groups will be described in the results section. As above mentioned, results will not be described in terms of conventional frequency bands, but rather in terms of the frequency ranges of those significant clusters that matched the adopted criteria of spatial and frequency adjacency.

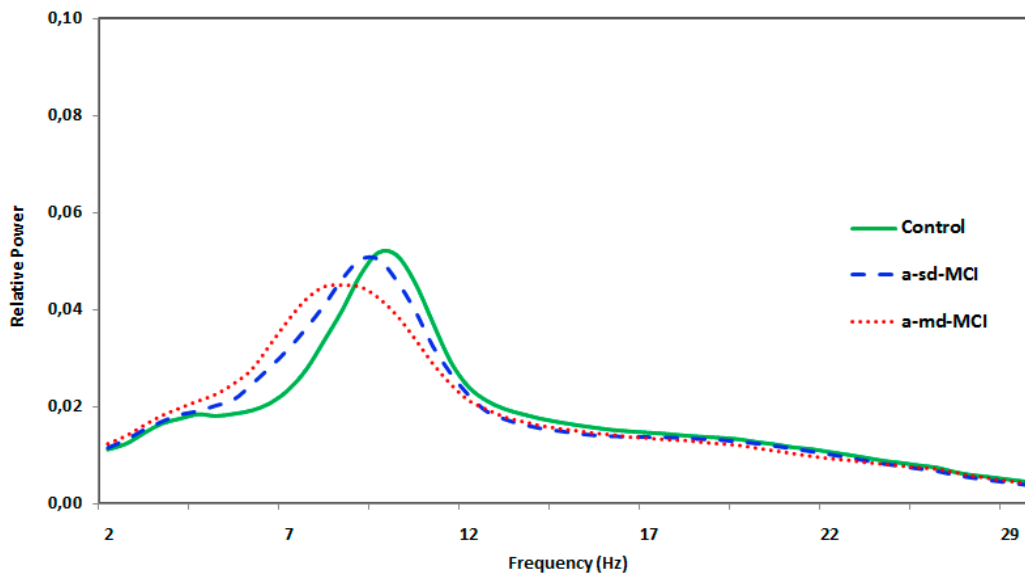
#### *2.5.1 Power, Hippocampal volumes and Neuropsychology Correlation*

First, a series of one-way ANOVA tests were performed in order to investigate the distribution of the neuropsychological scores and the hippocampal volumes among groups. In these analyses the variable “Diagnosis” (a-md-MCI, a-sd-MCI, Control) was considered the between groups factor. Then, the relationship between power values, hippocampal volumes and neuropsychological performance was assessed through Pearson correlation tests. The analyses were performed by correlating the averaged power on each resulting significant cluster of sensors in the t-test analyses (see below), and the neuropsychological scores on each test. In order to avoid the multiple comparisons problem, a permutation testing procedure was used (Nichols & Holmes, 2002). Five thousand surrogate correlation maps were calculated by randomly-distributing the combination of each power value and test score across subjects. The highest absolute value of the Pearson correlation coefficient obtained from each surrogate was retained in order to obtain an empirical null distribution of the statistic. Statistically significant thresholds were obtained from the quantiles of the distribution of these values. For example, the 95th quantile corresponded to a p-value of 0.05. This ensures there is only a 5% probability that one or more correlation values from the original statistical map would present differences above threshold due to spurious statistical fluctuations.

### 3. RESULTS

#### 3.1 Description of power spectra

First, only for descriptive purposes, we calculated the averaged relative power in the 1-30 Hz frequency range for each group (see Figure 2). It can be noticed that a power shift to lower frequencies was observed in MCIs, especially in a-md-MCI patients. Thus, the averaged relative power in a-md-MCI patients showed a frequency peak of about 8.5 Hz, while in a-sd-MCI subjects the peak appeared at about 9.5 Hz. Healthy controls showed their maximum value at about 10Hz. Moreover, the profile of the spectral distribution was quite different among groups. Both MCI groups (especially the a-md-MCI group) showed a broader spectral distribution in the 5-12 Hz frequency range, indicating a higher variability and a tendency to lower frequency peaks across subjects. On the contrary, the control group exhibited a narrower spectral distribution, indicating a lower variability and a tendency to frequency peaks that converge within the range of alpha band.

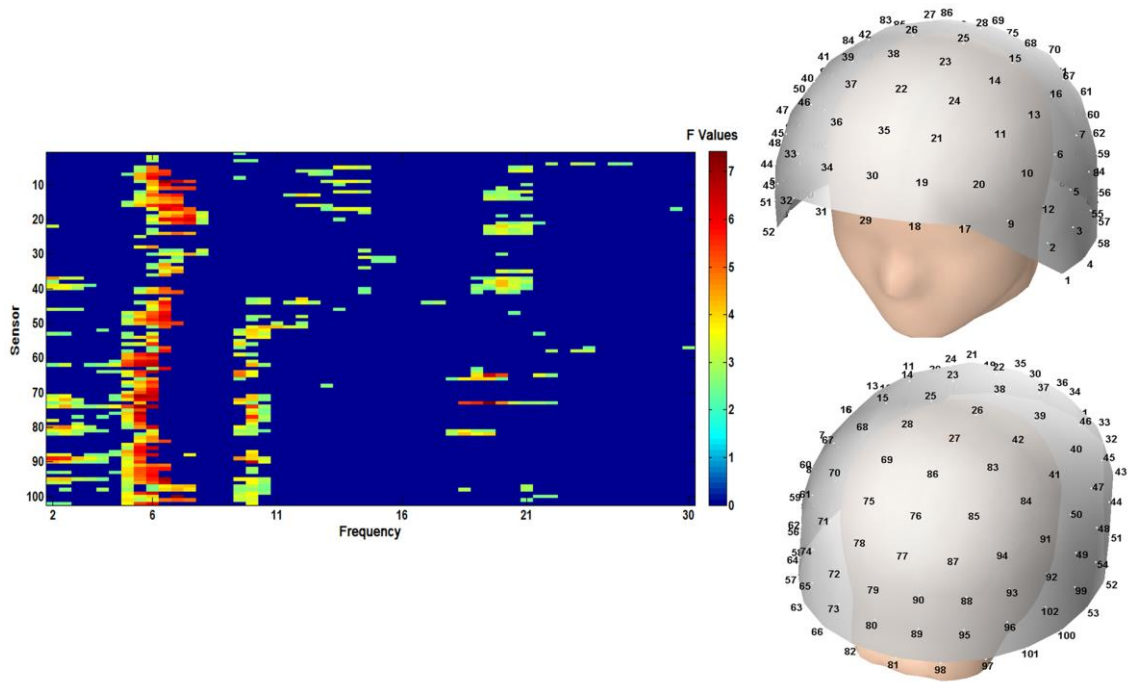


**Figure 2.** Average relative power spectra for all channels in the Control group (green line), the a-sd-MCI group (blue dashed line) and the a-md-MCI group (red dotted line). Spectra are represented in the “x” axis from 1 to 30Hz frequency band and relative power values in the “y” axis. It is very important to point out for the reader that in all figures the red color represents the a-md-MCI group, the blue color represents the a-sd-MCI group and the green color represents the control group.

### 3.2 Differences in relative power among groups

As previously mentioned, a series of exploratory ANOVA tests were calculated for each 0.5 Hz frequency step and sensor. The results of those ANOVAs are displayed in Figure 3. Overall, significant differences seem to converge within three frequency ranges: (1) a low-frequency range that includes frequencies between 2 and 8 Hz, (2) a range that includes frequencies between 9 and 12 Hz, and (3) a high-frequency range that includes frequencies between 16 and 23 Hz. These preliminary results might give us a hint about the frequency distribution of the significant clusters in the pairwise comparison.

In order to compare our results with previous MEG and EEG literature, significant clusters were referred in the range of the classical frequency bands (delta, theta...).

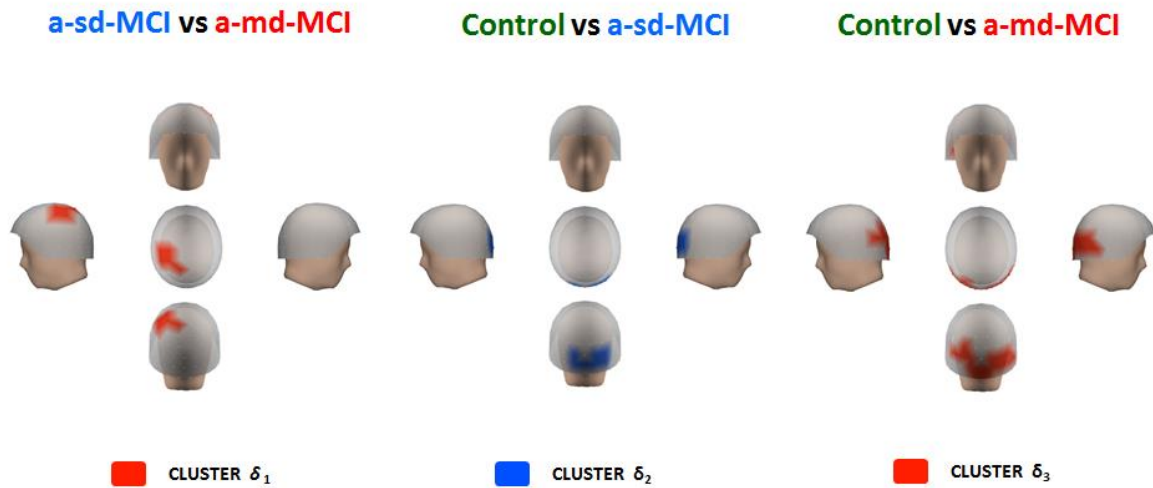


**Figure 3.** Significant F values corresponding to the exploratory ANOVA tests for each sensor and frequency step (left side). MEG helmet layout showing the distribution of sensors (right side).

#### 3.2.1 Differences within the delta band range

The a-md-MCI group showed a significant increase of activity within the delta range as compared with a-sd-MCIs and healthy controls (see Figure 4). Differences between a-md-MCIs and a-sd-MCIs ( $t = -3.331$ ;  $p < 0.001$ ) appeared within a frequency range of 2-4 Hz in a cluster of sensors located in left centro-parietal regions (henceforth called cluster  $\delta 1$ ). The significant differences between a-sd-MCIs and controls ( $t = -2.280$ ;  $p < 0.05$ ) emerged from cluster of

sensors also within the 2-4 Hz frequency range (cluster  $\delta_2$ ) located in occipital regions. Finally, significant differences between a-md-MCIs and controls ( $t = -3.123$ ;  $p < 0.001$ ) emerged within the same 2-4 Hz frequency range in a cluster ( $\delta_3$ ) with a broader occipito-temporal distribution as compared with cluster  $\delta_2$ . Importantly (see below), cluster  $\delta_3$  contains all sensors included in cluster  $\delta_2$ .

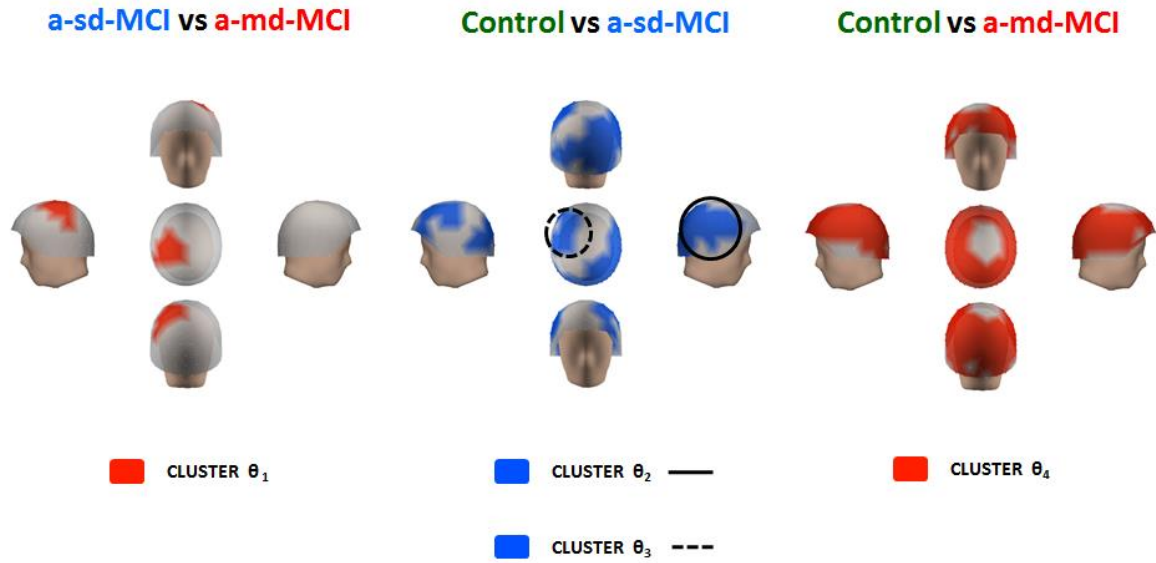


**Figure 4.** Differences within the delta band range. Red color indicates that the a-md-MCI group had more relative power than a-sd-MCI in left centro-parietal regions (cluster  $\delta_1$ ), and in occipito-temporal areas in comparison with the control group (cluster  $\delta_3$ ). Blue color indicates that the a-sd-MCI had more relative power in posterior regions than the control group (cluster  $\delta_2$ ).

### 3.2.2 Differences within the theta band range

Very similar to previous results, power within the theta range was significantly higher in the a-md-MCI group. The a-md-MCI group showed increased relative power ( $t = -2.007$ ;  $p < 0.05$ ) as compared with a-sd-MCIs within a 5-7 Hz frequency range in a cluster of sensors (cluster  $\theta_1$ ) located in left centro-parietal regions (see Figure 5). The a-sd-MCI group showed increased power within the typical 4-8 Hz range of theta band in two clusters of sensors (clusters  $\theta_2$  and  $\theta_3$ ) when compared with the control group. Cluster  $\theta_2$  ( $t = -2.939$ ;  $p < 0.005$ ) extended over most of the right lateral fronto-temporo-parieto-occipital region, while cluster  $\theta_3$  ( $t = -2.231$ ;  $p < 0.05$ ) had a left fronto-central location. When a-md-MCIs and controls were compared, the a-md-MCIs exhibited the same pattern of increased relative power within the 4-8 Hz range ( $t = -4.107$ ;  $p < 0.0001$ ), but in this case such differences emerged from a cluster that basically

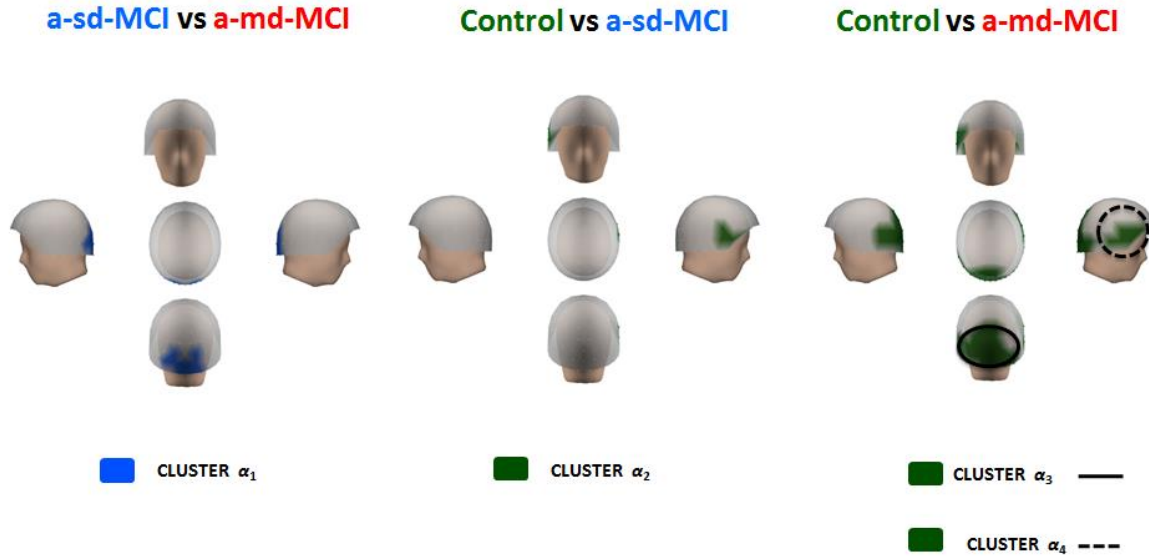
included all sensors excepting those located around the vertex (cluster  $\theta_4$ ). Consequently, cluster  $\theta_4$  encompassed sensors and frequency ranges in clusters  $\theta_1$ ,  $\theta_2$ , and  $\theta_3$  (see below).



**Figure 5.** Differences within the theta band range. Red color indicates that the a-md-MCI group exhibited more relative power in left centro-parietal regions than a-sd-MCI group (cluster  $\theta_1$ ) and in practically the whole head when compared with control group (cluster  $\theta_4$ ). The a-sd-MCI exhibited a significant power increase compared with the control group in two clusters of sensors, represented in blue color, that involved the right lateral fronto-temporo-parieto-occipital region (cluster  $\theta_2$ ) and the left fronto-central location (cluster  $\theta_3$ ).

### 3.2.3 Differences within the alpha band range

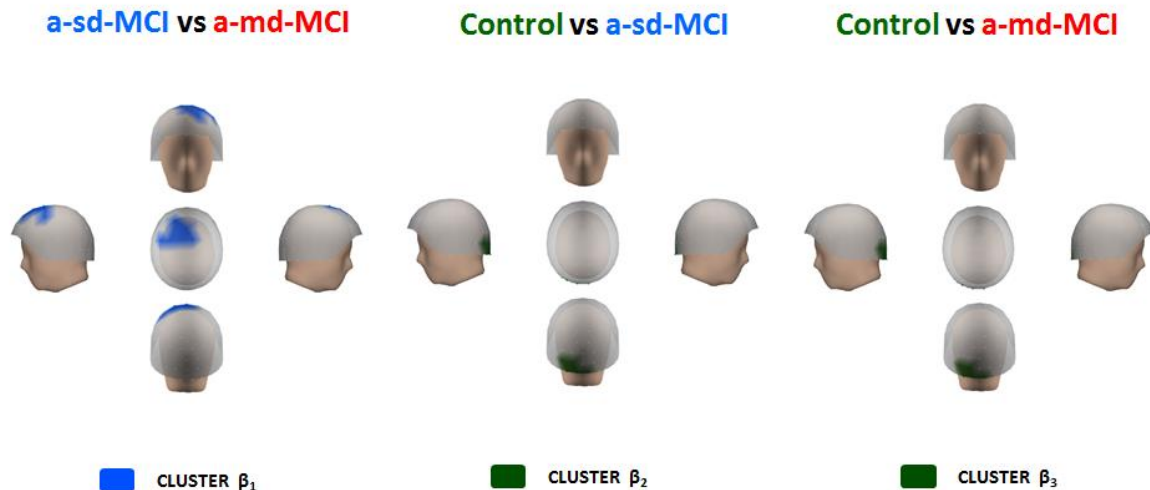
The a-md-MCI group (see Figure 6) showed reduced relative power values as compared to a-sd-MCIs in a small occipital cluster of sensors (cluster  $\alpha_1$ ) within a frequency range of 9 to 11 Hz ( $t= 2.457$ ;  $p< 0.01$ ). The a-sd-MCI group exhibited reduced relative power as compared to controls ( $t= 2.279$ ;  $p< 0.05$ ) within a 10-12 Hz frequency range in a small right fronto-temporal cluster of sensors (cluster  $\alpha_2$ ). Finally, a-md-MCIs showed significantly lower relative power values within a 8-12 Hz frequency range in two different clusters of sensors. Cluster  $\alpha_3$  ( $t= 2.752$ ;  $p< 0.001$ ) extended bilaterally over the occipital region and contained all sensors in cluster  $\alpha_1$  (see below). Cluster  $\alpha_4$  ( $t= 2.703$ ;  $p< 0.001$ ) extended over the right lateral fronto-temporal region and contained all sensors in cluster  $\alpha_2$ .



**Figure 6.** Differences within the alpha band range. The cluster in blue represents an increase in relative power within the a-sd-MCI group in occipital regions (cluster  $\alpha_1$ ), as compared with the a-md-MCI group. The control group showed more relative power in right fronto-temporal areas (cluster  $\alpha_2$ ), in bilateral occipital regions (cluster  $\alpha_3$ ), and in right lateral fronto-temporal regions (cluster  $\alpha_4$ ) than both MCI groups. These clusters are represented in green color.

### 3.2.4 Differences within the beta band range

As previously described for the alpha range, relative power within the beta range was significantly decreased in the MCI groups (see Figure 7). Of note, a-md-MCIs showed reduced relative power as compared with a-sd-MCIs ( $t = 2.262$ ;  $p < 0.05$ ) in a fronto-central cluster with left predominance (cluster  $\beta_1$ ) within a frequency range of 20 to 22 Hz. The a-sd-MCI group exhibited lower power values than controls within a broader frequency range of 16 to 23 Hz ( $t = 2.654$ ;  $p < 0.001$ ) in a small cluster of occipital sensors (cluster  $\beta_2$ ). Similarly, a-md-MCIs showed reduced power values as compared with controls ( $t = 2.126$ ;  $p < 0.05$ ) in the same 16-23 frequency range of 16 to 23 Hz and basically within the same occipital cluster of sensors observed in the controls vs. a-sd-MCI comparison (cluster  $\beta_3$ ).



**Figure 7.** Differences within the beta band range. Blue color indicates that the a-sd-MCI group exhibited increased relative power in a fronto- central cluster (cluster  $\beta_1$ ) as compared with a-md-MCI. In the control group there is an increased of relative power, indicated in green color, in occipital areas (clusters  $\beta_2$  and  $\beta_3$ ) when compared with a-sd and a-md-MCI groups.

### 3.3 Power, Hippocampal Volumes and Neuropsychology Correlations

As previously described, a series of exploratory one-way ANOVAs were performed to assess the differences in terms of neuropsychological tests performance and hippocampal volumes among groups. Firstly, the ANOVA revealed a significant effect of “Diagnosis” on LH\_ICV and RH\_ICV ( $p < 0.0001$ ) (see table 1). Post-hoc comparisons with Bonferroni correction showed significant differences for LH\_ICV and RH\_ICV between controls and MCIs ( $p < 0.001$ ), with larger hippocampal volumes within the healthy group. On the other hand, a-md-MCIs exhibited smaller volumes than a-sd-MCIs but the comparison failed to reach the level of statistical significance ( $p > 0.05$ ). A significant effect of “Diagnosis” was also found for all neuropsychological tests, with the exception of Clock Drawing Test, VOSP, and TMTA accuracy. This initial finding was further explored by means of post-hoc pair-wise comparison with Bonferroni correction. MMSE scores were significantly higher in control subjects as compared with both clinical groups ( $p < 0.001$ ), but in addition a-sd-MCI’s MMSE scores were significantly higher as compared to those of a-md-MCIs ( $p < 0.01$ ) (see table 1). Direct digit Span scores were significantly higher in controls, as compared with both clinical groups ( $p < 0.05$ ), but no significant differences emerged from the comparison of MCI subtypes. Identical results were obtained in the analysis of Inverse Digit Span, and Immediate and Delayed Recall. Phonemic Fluency scores were also significantly higher in controls when compared with both clinical groups ( $p < 0.05$ ), and a-sd-MCIs showed significantly increased fluency values than a-md-MCIs

( $p < 0.05$ ). Controls also exhibited increased semantic fluency values ( $p < 0.05$ ), but no significant differences were found between MCI subtypes. An identical pattern was found in the comparison of TMTA time, and TMTB accuracy and time scores. In both cases a-sd-MCI showed higher scores as compared to a-md-MCIs but the effect did not reach the significance level. Ideomotor Praxis values were also significantly higher in controls ( $p < 0.05$ ). Finally, BNT scores presented a slightly different behavior. First, no significant differences emerged from the comparison of controls and a-sd-MCI patients. However, controls showed higher scores than a-md-MCIs ( $p < 0.01$ ), and a-md-MCIs exhibited significantly higher scores than a-sd-MCIs ( $p < 0.05$ ). According to these results, it seems that both MCI groups are similarly impaired in memory tasks but a-md-MCIs tend to show a poorer performance in language tasks, especially in the BNT. Additionally, a-md-MCIs presented the lowest MMSE scores, indicating a significantly more impaired general cognitive status.

Once this exploratory analysis was carried out, and with the aim of investigating the relationship between neurophysiological activity, hippocampal atrophy and cognitive performance, averaged power values in the significant clusters were correlated with hippocampal volumes and neuropsychological test scores in the whole sample (a-md-MCIs + a-sd-MCIs + Controls). In order to avoid redundant information, and considering the high degree of overlapping among clusters, we decided to submit for correlation analyses only those clusters that matched the following criteria: (1) clusters without spatial or spectral coincidence with other clusters (i.e. clusters  $\delta 1$  and  $\beta 1$ ), and (2) broader clusters that encompassed the sensors and spectral ranges of smaller clusters (i.e.  $\delta 3$ ,  $\theta 4$ ,  $\alpha 3$ ,  $\alpha 4$ , and  $\beta 3$ ). Pearson's "r" and "p" values of all significant correlations are displayed in Table 2.



**Table 2.** Pearson correlation analyses of averaged power values in the significant clusters with neuropsychological test scores and hippocampal volumes in the whole sample. The table shows the “p” and “r” (correlation index) values that were significant ( $p < 0.05$ ).

Cluster	$\delta_1$	$\delta_3$	$\theta_4$	$\alpha_3$	$\alpha_4$	$\beta_1$	$\beta_3$
<b>MMSE</b>	p= 0,01115 r= -0,2528	p= 0,01201 r= -0,25033	p= 0,00032 r= -0,35269	n.s.	p= 0,00125 r= 0,31824	p= 0,03455 r= 0,21162	n.s.
<b>Inverse Digit Span</b>	p= 0,03296 r= -0,2114	p= 0,00078 r= -0,32741	p= 0,00700 r= -0,26550	p= 0,02979 r= 0,21526	p= 0,02038	n.s.	n.s.
<b>Immediate Recall</b>	n.s.	p= 0,00003 r= -0,39799	p= 0,000001 r= -0,45913	p= 0,00992 r= 0,25424	p= 0,00041 r= 0,34337	n.s.	p= 0,00331 r= 0,28818
<b>Delayed Recall</b>	n.s.	p= 0,00118 r= -0,32295	p= 0,000001 r= -0,49822	p= 0,01768 r= 0,23922	p= 0,00037 r= 0,35267	n.s.	p= 0,00083 r= 0,33234
<b>TMT A (acc.)</b>	p= 0,00417 r= -0,2827	n.s.	p= 0,03475 r= -0,21034	n.s.	n.s.	n.s.	n.s.
<b>TMT A (time)</b>	p= 0,03870 r= 0,2061	p= 0,01142 r= 0,25080	n.s.	n.s.	n.s.	n.s.	n.s.
<b>TMT B (acc.)</b>	n.s.	p= 0,02988 r= -0,22656	p= 0,00125 r= -0,33153	n.s.	n.s.	p= 0,01580 r= 0,25102	n.s.
<b>TMT B (time)</b>	n.s.	n.s.	n.s.	p= 0,01086 r= -0,26303	n.s.	n.s.	n.s.
<b>BNT</b>	n.s.	n.s.	p= 0,01214 r= -0,24996	n.s.	n.s.	p= 0,04702 r= 0,19912	n.s.
<b>Phonemic Fluency</b>	n.s.	p= 0,00078 r= -0,32889	p= 0,02630 r= -0,22109	p= 0,02740 r= 0,21953	n.s.	n.s.	n.s.
<b>LH_ICV</b>	n.s.	n.s.	p= 0,0032 r= -0,3196	n.s.	p= 0,0022 r= 0,2501	n.s.	p= 0,0235 r= 0,2485
<b>RH_ICV</b>	n.s.	n.s.	p= 0,0022 r= -0,3309	n.s.	p= 0,0533 r= 0,2129	n.s.	p= 0,0003 r= 0,3882

Within the delta band range, cluster  $\delta_1$  was inversely correlated with MMSE, Inverse Digit Span, and TMTA accuracy, indicating that higher scores in this cluster are associated with a lower cognitive status. Cluster  $\delta_1$  was positively correlated with TMTA time. Cluster  $\delta_3$  was inversely correlated with MMSE, Inverse Digit Span, Immediate and Delayed Recall, Phonemic Fluency, and TMTB accuracy. Mirroring cluster  $\delta_1$ , cluster  $\delta_3$  was also directly correlated with TMTA time.

Cluster  $\theta 4$  was inversely correlated with both hippocampus and several tests: MMSE, Inverse Digit Span, Immediate Recall, Delayed Recall, Rule Shift Cards, Phonemic Fluency, TMTA and TMTB accuracy, and BNT. On the other hand, cluster  $\theta 4$  was directly correlated with TMTA and TMTB time. The pattern of correlations within the low-frequency domain clearly suggests that the higher activity within delta and theta ranges, the poorer cognitive performance in several domains.

Cluster  $\alpha 3$  was directly correlated with Immediate and Delayed Recall, Inverse Digit Span, and Phonemic Fluency, indicating that higher posterior activity within the alpha range is associated with a better cognitive performance. Cluster  $\alpha 3$  was inversely correlated with TMTB time. Supporting this idea, cluster  $\alpha 4$  was also positively correlated with both hippocampus, MMSE, Inverse Digit Span, and Immediate and Delayed Recall.

Finally, within the beta range, cluster  $\beta 1$  was directly correlated with MMSE, BNT and TMTB accuracy. Cluster  $\beta 3$  was positively correlated with both hippocampal volumes, Immediate and Delayed Recall. This correlation pattern suggests that an enhanced activity within the high-frequencies range is associated with a lesser hippocampal atrophy and a better cognitive performance.

Lastly, in order to explore the relationship between hippocampal volumes and neuropsychological scores, both measures were correlated. LH\_ICV was positively correlated with MMSE ( $p = 0,0040$ ;  $r = 0,3832$ ), Immediate Recall ( $p = 1,8 \times 10^{-6}$ ;  $r = 0,4992$ ), Delayed Recall ( $p = 6,2 \times 10^{-10}$ ;  $r = 0,5941$ ), VOSP ( $p = 0,0212$ ;  $r = 0,2558$ ), TMT B (accuracy) ( $p = 0,0430$ ;  $r = 0,2328$ ) and negatively with TMT B (time) ( $p = 0,032$ ;  $r = -0,2446$ ). Similarly, RH\_ICV was directly correlated with MMSE ( $p = 0,0018$ ;  $r = 0,3413$ ), Immediate Recall ( $p = 0,8 \times 10^{-5}$ ;  $r = 0,4709$ ), Delayed Recall ( $p = 3,93 \times 10^{-7}$ ;  $r = 0,5314$ ), Rule shift Cards ( $p = 0,0422$ ;  $r = 0,2292$ ), TMT B (accuracy) ( $p = 0,0164$ ;  $r = 0,2745$ ) and inversely correlated with TMT B (time) ( $p = 0,0064$ ;  $r = -0,3081$ ). These findings confirm the relationship among the hippocampal atrophy, the neurophysiological changes and the global deficit observed in different cognitive domains, such as memory, perception or executive functioning.

#### 4. DISCUSSION

We are aware that the clusterization of the frequency bands done in this study rendered difficult the comparison of our findings with the previously published literature. For this reason, we decided to compare the current findings with the classical frequency band closer to the frequency-cluster found in this study.

The results of the present study basically support previous reports about EEG/MEG spectral analysis in MCI, and add new information on the spectral profiles of MCI subtypes that was not previously addressed. As it might be expected, MCI patients (especially the a-md-MCI group) showed a generalized power increase within the theta range accompanied by a power decrease within the alpha and beta ranges in the posterior regions of the brain. More importantly, a-md-MCIs exhibited increased power within the delta and theta ranges as compared with a-sd-MCIs. This tendency (see Figure 2) is a new example of the “shift to the left” in the spectral profile observed in AD and MCI patients that correlates with the degree of cognitive impairment (Rodriguez et al. 1999; Fernández et al., 2006a).

From the last years, there is evidence of hyperactivation (Dickerson et al., 2005; F Maestú et al., 2008) or hypersynchronization (Bajo et al., 2010) of different regions of the brain in patients with amnesic MCI in comparison to healthy controls but as well to AD patients. This increase is an indication of compensatory activity due to the loss of efficiency of the memory networks that leads to the idea of a non-clear linear neurophysiological process between healthy aging and dementia. In addition, there are some biochemical (Lavenex & Amaral, 2000) or resting state functional studies (Osipova et al., 2006) that suggest that the differences between controls and MCI patients seem not to be so linear as our present results. Nonetheless, overall our findings seem to confirm the hypothesis of a spectral pattern more proximate to the typical AD-profile, mainly in a-md-MCIs. Previous EEG (Babiloni et al., 2004; Dauwels et al., 2011; Huang et al., 2000; Jeong, 2004) and MEG studies (Berendse, Verbunt, Scheltens, van Dijk, & Jonkman, 2000; Fernandez et al., 2002; Fernández et al., 2003, 2006a) of AD found an increased power in the low frequency bands (delta and theta), accompanied by a decreased power in the high-frequencies range (alpha, beta and gamma). Such pattern of spectral changes is a consistent finding that correlates with cognitive performance and functional status (Fernandez et al., 2002; Pritchep et al., 1994; van Deursen, Vuurman, Verhey, van Kranen-Mastenbroek, & Riedel, 2008). More specifically, in a previous study of our group (Fernandez & Hornero, 2006b) Fernandez et al. 2006a) AD patients were characterized by a power increase within the 2-4Hz frequency range, accompanied by a power decrease within

the 16-28Hz frequency range that basically parallels the spectral changes observed in the present investigation of MCI patients.

The sequence of spectral changes in AD is assumed to start with an increase of theta and a decrease of beta activity, which are followed by a decrease in alpha activity. Delta power is usually believed to increase only in more severe stages of the disease (Jeong 2004). However, several studies also reported an increase of delta power in MCI patients when compared with healthy aged controls (see for example Babiloni, Benussi, et al., 2006a; Babiloni, Binetti, et al., 2006b; Rossini et al., 2008; Babiloni et al., 2010). Moreover, Fernandez and coworkers (Fernández, Turrero, et al., 2006c; Fernandez et al. 2006c) demonstrated that MCI patients with elevated delta activity in posterior parietal regions had a significantly elevated risk of conversion to AD. This line of evidence indicates that very similar patterns of neurophysiological activity can be found in MCI patients and AD patients, supporting the idea of some degree of overlapping (Fernandez et al. 2006a). An increase of low-frequency activity is not the only shared characteristic. MCI and AD share neuropathological and functional features, including: tau and ApoE abnormal proteins (Mufson et al., 1999); reduced hippocampal and temporal lobe volumes (Clifford R Jack, Petersen, Xu, & Al, 1999; S. B. Martin, Smith, Collins, Schmitt, & Gold, 2010); temporo-parieto-occipital hypometabolism-hypoperfusion in PET or SPECT scans (Nestor et al., 2004) and cholinergic dysfunction (Haense et al., 2012).

The “proximity” of a-md-MCIs’ spectral profiles to the typical AD pattern, together with the significantly lower MMSE, phonemic fluency and language scores within this group (see Table 1), may be interpreted as a sign of greater deterioration in these patients. This affirmation is supported by the correlations between neurophysiological, structural, and neuropsychological data. The a-md-MCI group showed the highest levels of activity within delta and theta ranges, and such activity was inversely correlated with MMSE scores and hippocampal volumes (in this case only theta range power). In addition, activity within delta and theta ranges was inversely correlated with the performance on tests of memory, language, attention and executive functions such as Immediate and Delayed Recall, Inverse Digit Span, TMTA and TMTB, BNT, etc. The correlation pattern of clusters within alpha and beta ranges was also illustrative. The a-md-MCI group exhibited the lowest power within the alpha and beta ranges, especially in posterior sites, compared with the control group. Relative power values within the alpha range were directly correlated with hippocampal volumes and scores of Immediate and Delayed Recall, Inverse Digit Span, etc. Similarly, an increased activity within the beta range was positively correlated with hippocampal volumes and global cognitive status (i.e. MMSE) but

also with the performance on tests of memory, language, and executive functioning. Overall, a-md-MCIs exhibited the highest power values in the low-frequency range, the lowest power values in the high-frequency range, the poorer cognitive performance, and the smaller hippocampal volumes.

The correlation between spectral profiles and cognitive performance was previously assessed in several studies. For example, in their classic study Jelic et al. (1996) reported negative correlations between relative theta power, visuospatial function, memory and attention. More recent investigations, showed very similar tendencies. Van der Hiele et al. (2007) found negative correlations between theta power and tests of global domain. Also, negative correlations with tests such as the TMTB or Semantic Fluency were described by the authors. Babiloni et al. (2010) described negative correlations between delta power, Stroop and Digit-Symbol tests; while alpha 1 power was directly correlated with these neuropsychological measures. Although these investigations assessed the correlation between spectral and neuropsychological data, the importance of the regional distribution was not explicitly explored. It is noteworthy that the most significant difference between a-md-MCIs (i.e. patients with the poorest cognitive performance and lowest mental status) and a-sd-MCI patients appeared in fronto-central region (cluster  $\delta 1$ ). The implication of more anterior sites is associated with a posterior-to-anterior tendency of neurophysiological abnormalities, such as focal delta activity, observed with the progress of the disease (Fouquet et al., 2009; Osipova et al., 2005).

As in some previous studies (He et al., 2009; Whitwell et al., 2007; Zhang et al., 2012), where a-MCI subtypes were compared, we failed to find significant statistical differences in hippocampal volumes. However, in a follow-up study, Tabert et al. (2006) found that 38 out of 39 patients that converted to AD had a base-line diagnosis of a-md-MCI, while none of them had a base-line diagnosis of a-sd-MCI. Diniz, Nunes, Yassuda, & Forlenza (2009) found that those MCI who progress to dementia showed a worse global cognitive performance and multiple cognitive deficits at baseline than those MCI who remained stable. Similarly, Han et al. (2012) reported that the rate of progression to dementia in a-sd-MCI patients was very similar to the rate of reversion to the normal cognition, while in the a-md-MCI group the rate of conversion to dementia was significantly higher when compared to the rate of reversion. Interestingly, the authors interpreted these findings as a sign of broader cerebral “degeneration” in the a-md-MCI group. Brodaty et al.’s (2012) findings mirrored those reported by Han and coworkers.

The relationship between elevated risk of conversion and presence of neuropathological signs has been established in a-md-MCI patients. For instance, Caffarra, Ghetti, Concari, & Venneri (2008) accomplished a follow-up study where a-sd-MCI, a-md-MCIs and “dysexecutive MCI” patients underwent SPECT evaluation at base-line. Both amnesic groups showed significant hypoperfusion in the left hippocampus and parahippocampal gyrus, but a-md-MCIs showed an additional hypoperfusion in left posterior cingulated gyrus. Notably, the 55% of a-md-MCIs progressed to AD in a maximum follow-up period of 24 months, while none of the a-sd-MCI patients progressed to dementia. These results might suggest that, contrary to the classical understanding of a-MCI as a risk factor for dementia, the a-sd-MCI subtype is in fact a “benign” form of MCI. This challenging idea was posed by Nordlund et al. (2010) in an excellent investigation. The authors evaluated the two-year outcome in a sample composed of a-sd-MCIs, a-md-MCIs, na-sd-MCIs, and na-md-MCIs. In addition, CSF samples were collected to estimate tau levels at baseline. Paralleling previous studies, only multidomain cases progressed to dementia, but more specifically 18 out of 21 patients that progressed to dementia had a baseline diagnosis of a-md-MCI. In fact, elevated tau levels in CSF and a diagnosis of a-md-MCI were the best predictors of progression to dementia. Nordlund et al.’s results further confirm Wolk et al.’s (2009) findings of elevated levels of AD-pathology in a-md-MCIs, in this case represented by a significantly higher number of PIB positive cases that exhibited an increased risk of conversion to AD.

At this point, three lines of evidence converge: (1) a-md-MCI and AD biological markers seem to overlap in a significant percentage of cases; (2) several studies support the notion of a more severe brain deterioration with a more frequent presence of AD-pathology and an elevated risk of conversion to AD in a-md-MCI subtype; and (3) our results indicate that a-md-MCIs exhibit a spectral profile very similar to that observed in AD which is associated with a more severe cognitive deterioration. Taken together, these lines of evidence lead us to discuss the notion of a-md-MCI as an independent clinical condition. Recently, in a key position article Dubois et al. (2010) revised the definition of AD and some related disorders. According to the new perspective proposed by the authors, some patients previously described as having MCI should be actually considered as suffering from “prodromal” AD. This is to say they already have AD. The category of prodromal AD encompasses all patients previously considered as having MCI who show positive biomarker evidence of AD-pathology in their brains. The concept of MCI only remains to define those patients that do not show biomarker evidence of AD-pathology.

According to previous literature and our own results, a-md-MCIs might be more likely to fulfill the criteria of prodromal AD proposed by Dubois et al. (2010). Nevertheless, the more important question addressed by our investigation is the potential role of neurophysiological techniques (EEG or MEG) as markers for AD-pathology. In a very recent study of our group (Fernández et al., 2013) it was demonstrated that delta current densities in posterior parietal, occipital, prerolandic and precuneus cortices distinguished between MCI patients, AD patients with different severity scores, and controls. More importantly, an increase of delta activity in posterior regions such as the right posterior parietal cortex and the precuneus indexed the transition from MCI to mild and from mild to more severe dementia. Considering the close relationship between cholinergic inputs and neurophysiological activity, we proposed that MEG spectral mapping might be a serious candidate for a “neural degeneration” marker of AD reflecting dysfunctional synaptic transmission. The new results presented here might support the role of MEG spectral analysis in the investigation within the healthy aging-AD continuum, particularly as an objective marker of disease progression associated with cognitive deterioration. Notwithstanding, the confirmation of such role should be attained by correlating MEG spectral data with CSF or imaging markers of AD. Also, the spectral information should be evaluated in individuals with elevated risk of developing AD such as the APOE4 carriers. According to our results, we hypothesize that patients with a diagnosis of a-md-MCI showing an exaggerated activity within the delta and theta ranges are cases of prodromal AD (according to Dubois et al.’s definition) with an elevated risk of rapid progression to a fully declared dementia. If this strong hypothesis is confirmed, MEG spectral analysis might serve as a quantitative measure to define the neurophysiological characteristics of prodromal AD.

## **STUDY II**

**Synchronization during an Internally Directed Cognitive State in healthy aging and Mild Cognitive Impairment. A MEG study**



## **1. AIMS AND HYPOTHESIS**

The present study evaluates functional connectivity patterns in MCIs and their age-matched controls during resting state and an internally-governed memory task with two levels of difficulty. The novelty of this study lies in its task design, which is a combination between resting state and a cognitive state with no external stimuli, being the self-monitoring required to performing it. This condition could be named “Internally Directed Cognitive State” (IDICS), and would allow us to explore how the brain works during a pure cognitive state. For this purpose we have chosen a calculation task, since it can be used in an internal way and it is usually impaired in AD (Parlato et al., 1992; Rémy et al., 2004) and MCI patients (Li et al., 2010; L Zamarian et al., 2007; Laura Zamarian et al., 2007). The performance of this task requires the knowledge of numbers and arithmetic (long term memory), the ability to manipulate and update the information mentally (working memory), a high level of self-monitoring and the capacity to inhibit distracting stimuli (executive functioning). Therefore, the principal aim of this study was to compare the connectivity patterns across groups and conditions.

The exploration of the neural networks involved during a pure resting state and IDICS in MCI and controls was proposed under the following hypotheses: 1) During resting state, the MCI group will show a decreased synchronization in higher frequency bands compared to the control group 2) Our results will differ from those studies which have employed external stimuli since IDICS requires higher levels of internal information processing and therefore of top-down control 3) MCI patients will show a higher inter and intra hemispheric synchronization than the control group during the execution of the task 4) In both groups, the overall connectivity will increase along with the difficulty of the task.

## 2. MATERIALS AND METHODS

### 2.1 Subjects

89 subjects older than 65 years enrolled voluntarily in the study. They were all right handed (Oldfield, 1971), and native Spanish speakers. The whole sample comprised 34 healthy elders and 55 MCI patients. For the subsequent analysis, only 38 MCIs (18 males) and 32 controls (10 males) were used: 17 MCIs and 2 controls were excluded based on task execution (see *Experimental design*). Healthy individuals were recruited from the “Seniors Center of the district of Chamartín, Madrid”, and MCI patients from the Geriatric and Neurological Units of the “Hospital Universitario San Carlos, Madrid” and the “Memory Decline Prevention Center, Madrid”.

Subject’s characteristics are shown in Table 1. No statistical differences ( $p < 0.10$ ) were found between Controls and MCIs in age, sex or educational level, but both groups did differ in MMSE scores and normalized hippocampal volume ( $p < 0.00001$ ). Normalized hippocampal volume in MCI was significantly smaller than in controls, which is a biomarker that reflects neuronal injury (M. S. Albert et al., 2011).

### 2.2 Diagnostic criteria

All participants were rated with a variety of standardized diagnostic instruments that included: the Spanish version of the MMSE (Lobo et al., 1979), the Geriatric Depression Scale (GDS; Yesavage et al., 1982), the Global Deterioration Scale (GDS; Reisberg et al., 1982), the Hachinski Ischemic Score (HIS; Rosen et al., 1980), the Functional assessment questionnaire (FAQ; Pfeffer et al., 1982), the questionnaire for Instrumental Activities of Daily Living (IADL; Lawton & Brody, 1969), and the Functional Assessment Staging (FAST; Auer & Reisberg, 1997).

MCI diagnosis was established according to Petersen’s criteria (Petersen 2004; Grundman et al. 2004): (1) memory complaint, corroborated by an informant; (2) abnormal memory function, documented by delayed recall of one paragraph from the Logical Memory II subtest of the Wechsler Memory Scale–Revised (cutoff scores  $\leq 16$  for  $\geq 16$  years of education;  $\leq 8$  for  $\geq 8$ –15 years of education); (3) normal general cognitive function, determined by a MMSE score greater than or equal to 24; (4) no or minimal impairment in activities of daily living (ADLs) revealed by the Lawton scale, as determined by a clinical interview with the patient and informant; and (5) not being sufficiently impaired, cognitively and functionally, to meet National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s

Disease and Related Disorders Association criteria (NINCDS-ADRDA) (G. McKhann et al., 1984) for AD as judged by a clinician experienced in AD research. MCIs were classified as amnesic MCI, at the stage 3 of the GDS and they did not meet the diagnostic criteria for dementia.

The age of the participants ranged from 65 to 85, since the incidence and prevalence of AD starts to rise around 65 years and at age of 80 and 90 it is nearly 50% (Zamrini et al., 2011). All of them were in good health, with no significant cerebral vascular disease, and no history of psychiatric or neurological disorders. Other inclusion criteria included a modified Hachinski score less than or equal to 4, a Geriatric Depression Scale score lower than 9 and a computed tomographic or magnetic resonance imaging (MRI) brain scan within 12 months before MEG screening without indication of infection, infarction, or focal lesions. We performed on every participant complementary explorations (Class II evidence level) to rule out possible causes of cognitive decline such as B12 vitamin deficit, diabetes, thyroid problems, syphilis, or human immunodeficiency virus. Besides, those subjects with alcoholism (>3 alcoholic beverages per day) or chronic use of medication such as anxiolytics, were not included in the study. Finally, MCI patients were required to suspend the ingestion of drugs which could affect MEG activity (e.g. cholinesterase inhibitors) 48 hours before the MEG recordings.

Patients and controls also received a neuropsychological assessment, in order to explore their cognitive status in multiple cognitive functions such as memory, language and executive functions. This included: Clock Drawing Test (Agrell & Dehlin, 1998), Direct and Inverse digit Span Test (Wechsler Memory Scale Revised, WMS-III; Wechsler, 1987), Immediate and Delayed Recall (WMS-III; Wechsler, 1987), Phonemic and Semantic Fluency (Controlled Oral Word Association Test, COWAT, Benton & Hamsher, 1989), Ideomotor Praxis of Barcelona Test (Peña-Casanova, 1990), Rule shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS; Norris & Tate, 2000), Visual Object and Space Perception Test (VOSP; Warrington & James, 1991), Boston Naming Test (BNT; Kaplan et al., 1983) and Trail Making Test A and B (TMTA and TMTB; Reitan, 1958)

Before the MEG recording, all participants gave a written informed consent to participate in the investigation. The study was approved by the local ethics committee.

**Table 1.** Subject's information. Characteristics given as mean  $\pm$  standard deviation. M = males, F = females, MMSE =Mini Mental State Examination score. Educational level was grouped into five levels: 1: illiterate, 2: primary studies, 3: Elemental studies, 4: High school studies, 5: University studies. P-values for between-groups differences were introduced. Wilcoxon-Mann-Whitney test was used for continuous variables (age, educational level, MMSE, volume ratios) and Fisher's exact test for sex differences.

	Control group (n=32)	MCI group (n= 38)	p-values
<b>age (years)</b>	71.8 $\pm$ 3.8	72.5 $\pm$ 4.5	0.50
<b>sex (M/F)</b>	10 / 22	18 / 20	0.22
<b>educational level</b>	3.8 $\pm$ 1.1	3.3 $\pm$ 1.3	0.12
<b>MMSE score (points)</b>	29.3 $\pm$ 0.9	27.1 $\pm$ 2.2	3.9 $\cdot$ 10 <sup>-5</sup>
<b>volume ratio:</b>			
<b>hippocampus / intracranial</b>	(5.09 $\pm$ 0.62) $\cdot$ 10 <sup>-3</sup>	(4.17 $\pm$ 0.89) $\cdot$ 10 <sup>-3</sup>	9.8 $\cdot$ 10 <sup>-5</sup>
<b>Number of clean 4-second segments</b>	<b>resting</b> (27.6 $\pm$ 6.5)	(27.9 $\pm$ 5.6)	0.83
	<b>1-subtraction</b> (22.5 $\pm$ 6.8)	(21.7 $\pm$ 8.2)	0.55
	<b>3-subtraction</b> (21.2 $\pm$ 5.6)	(21.9 $\pm$ 8.2)	0.94

### 2.3 Experimental design

IDICS was based on a calculation mental task, which consisted of subtracting numbers mentally. Participants were told an initial number, and instructed to repeatedly subtract a given amount from that number over the course of one minute. After that minute, they were asked what number they had reached, and the researchers made a note of their answers. Participants were allowed to perform the subtraction at their own pace and were instructed to keep their eyes closed while performing the task. Counting aloud and finger counting was prohibited, and participants were instructed to continue the task regardless of any doubts of error.

The IDICS task had two levels of difficulty:

- 1-subtraction: Participants had to subtract 1 by 1 from a given fixed number.
- 3-subtraction: Participants had to subtract 3 by 3 from a given fixed number.

In each subtraction condition, four numbers were given: two two-digit and two three-digit. The same numbers were used for all the participants but their order was changed in order to counterbalance experimental conditions. So, for each subject we registered 8 minutes of brain activity and 8 behavioral responses, one for each given number. If a subject performed badly more than one subtraction exercise in any condition (1-subtraction of 3-subtraction), he/she was excluded from the present functional connectivity analysis. Following this criterion, 17 MCIs and 2 controls were excluded and only data from 70 subjects out of 89 recorded subjects were used. A subtraction exercise was classified as badly performed if (1) participants informed that they got lost or stopped performing the task correctly (2) they summed instead of subtracting or (3) the final number they reached seemed out of a normal range (either too big meaning they had subtracted very slowly or stopped doing so at some point or too small meaning they had probably skipped some number intervals). As the task is not externally controlled except for the number they give after one minute, the external assessment of the performance is rather qualitative, and the validity of the data relied to a great extent on the participants' assessment of their own performance.

#### *2.4 MEG Recordings and preprocessing*

Brain magnetic fields were measured while each subject sat in a magnetically shielded room (VacuumSchmelze GmbH, Hanau, Germany), using a 306 channel Vectorview system (Elekta Neuromag) at the Center for Biomedical Technology (Madrid, Spain). Fields were measured during a task-free, 3 min eyes-closed condition, and during the execution of the IDICs task. Subjects were instructed to close their eyes in both conditions and move as little as possible.

The MEG system comprised 102 magnetometers and 204 planar gradiometers. Sampling frequency was 1000Hz and an online filtering of 0.1-330Hz was applied. A head position indicator (HPI) system and a three-dimensional digitizer (Fastrak Polhemus) were used to determine the position of the head with respect to the sensor array. Four HPI coils were attached to the subject (one on each mastoid, two on the forehead), and their position with respect to the 3 fiducials (nasion, left and right pre auricular points) was determined. To record vertical electrooculogram (EOG), two electrodes were attached above and below the left eye, and a third one to the left earlobe for electrical grounding.

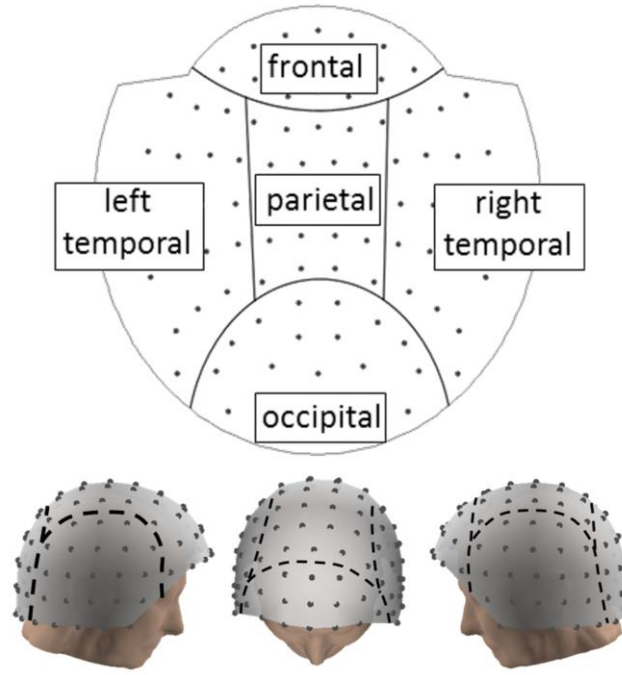
External noise was removed from the MEG data using the temporal extension of Signal-Space Separation (tSSS) (Samu Taulu & Kajola, 2005) as implemented with the MaxFilter software (version 2.2, Elekta-Neuromag) with a 10s raw data buffer and a subspace correlation limit of

0.9. Data was subsequently adjusted for head movement every 200 ms and transformed into a common space.

Data was then preprocessed with Fieldtrip (Oostenveld et al., 2011). The continuous time series (resting state and mental calculation task) were first separated into segments of 4 seconds. Then, jump, muscle and ocular artifacts were located using all 306 sensors and the additional EOG channels. All segments containing artifacts were eliminated from the analysis. As a result, all subjects had at least N=15 clean segments per condition. The amount of clean trials was similar across groups (Table 1).

### *2.5 Functional connectivity analysis*

In order to provide additional information to the measures widely used in the study of functional connectivity in dementia (coherence and synchronization likelihood), Phase Locking Value (PLV) (Mormann, Lehnertz, David, & E. Elger, 2000) between pairs of sensors was computed. To avoid mixing signals of different nature and noise profiles we used only magnetometers for this functional connectivity analysis (we note however that gradiometers were used in the temporal signal space separation step, so the resulting magnetometer data contain indirectly gradiometer information). We discarded 8 inferior temporal sensors from the analysis for being noisy, keeping 94 magnetometers for the analysis. Fig. 1 shows the distribution of these 94 channels in the MEG helmet in two and three dimensions, and separates channels into different head regions for better understanding of the geometry.



**Figure 1.** MEG sensor layout. Magnetometer positions in 2D and 3D, separated into frontal, parietal, occipital and left and right temporal regions

First, time series were filtered with a Finite Impulse Response filter of order 600 (which introduced a zero-phase distortion) into different frequency bands: delta (2-4Hz), theta (4-8Hz), alpha (8-12Hz), beta (12-30Hz), and gamma (30-45Hz), yielding a narrow band signal  $x_j(t)$  for each sensor  $j=1...102$ . Its phase  $\varphi_j(t)$  was extracted via a Hilbert Transform.

$$z_j(t) = x_j(t) + i \cdot \text{hilbert}(x_j(t)) = A_j(t) \cdot e^{i\varphi_j(t)} \quad (1)$$

Then, the synchronization between a pair of phases  $\varphi_j(t)$  and  $\varphi_k(t)$  was assessed by computing the PLV.

$$PLV = \frac{1}{M} \left| \sum_{m=1}^M e^{i(\varphi_j(t_m) - \varphi_k(t_m))} \right| \quad (2)$$

where  $M=4000$  is the number of samples in the time series (4 seconds sampled at 1000 Hz). This calculation was repeated for all pairs of sensors and averaged over segments belonging to the same condition, ending up with a symmetrical 94x94 connectivity matrix for each subject, condition (resting, 1- and 3-subtraction task) and frequency band.

## *2.6 Hippocampal volume*

Hippocampal volumes were measured in order to provide an anatomical measure that quantifies the degree of brain atrophy. Indeed a decrease in hippocampal volume has been related to MCI and Alzheimer's disease (Dubois et al., 2007; D. V Moretti et al., 2007). For each subject, a high resolution T1 weighted magnetic resonance was acquired with a General Electric 1.5 Tesla magnetic resonance (MR) scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). Freesurfer software (version 5.1.0) and its specialized tool for automated subcortical segmentation (Fischl et al., 2002) were used to segment the subject's T1-weighted volume into different regions. Then, hippocampal volume was normalized with the overall intracranial volume to account for differences in head volume over subjects.

## *2.7 Statistical Analysis*

To compare connectivity values between conditions and groups (controls vs MCIs) we employed non-parametric statistical tests: Wilcoxon signed rank tests for differences between conditions and independent samples Wilcoxon-Mann-Whitney tests for differences between diagnoses. The results were corrected for multiple comparisons with a permutation technique that was introduced by (Maris & Oostenveld, 2007). Following this approach, elements from the two compared groups (e.g. condition 1 vs. condition 2 or diagnosis 1 vs. diagnosis 2) were shuffled randomly. Then, two subsets with the same size as the originals groups were created, that contained the shuffled elements. A new statistical test (signed rank Wilcoxon or Wilcoxon-Mann-Whitney) was performed for these two randomly chosen groups. This procedure was repeated 5000 times, yielding a set of surrogate p-values. The final and corrected p-value was then defined as the proportion of permutations with a p-value lower than the one in the original test.

To assess the relationship between neurophysiological activity and cognitive performance, the average connectivity in significant links in the between-conditions analysis was correlated with several neuropsychological test scores in the whole sample (controls and MCIs). Additionally, hippocampal volumes were also included for correlations, in order to have an anatomical measure of the pathology. Tests were described in the diagnostic criteria section of this paper. Spearman's  $r$  was calculated, along with the p-value testing for the hypothesis of no



correlation. This p-value was corrected for multiple comparisons with a permutation approach, as explained in the previous paragraph.

### 3. RESULTS

#### 3.1 Behavioral performance

In order to check whether subjects were actually performing the task after every 1-minute subtraction exercise, subjects announced the final number they had reached. Since all participants used the same original numbers in their countdown, the final number provides insight into the individuals' performance. The final numbers were compared between groups with a Wilcoxon-Mann-Whitney test. Significant differences in this final number would indicate a different execution level across groups. This comparison was done separately for all subtraction exercises (4 exercises for each subtraction task).

There were no significant differences between controls and MCI in any of the eight answers, although the MCI group's responses had higher standard deviation values than the Control's ones. This indicates that both groups were actively engaged in the task and had a similar performance (see table 2).

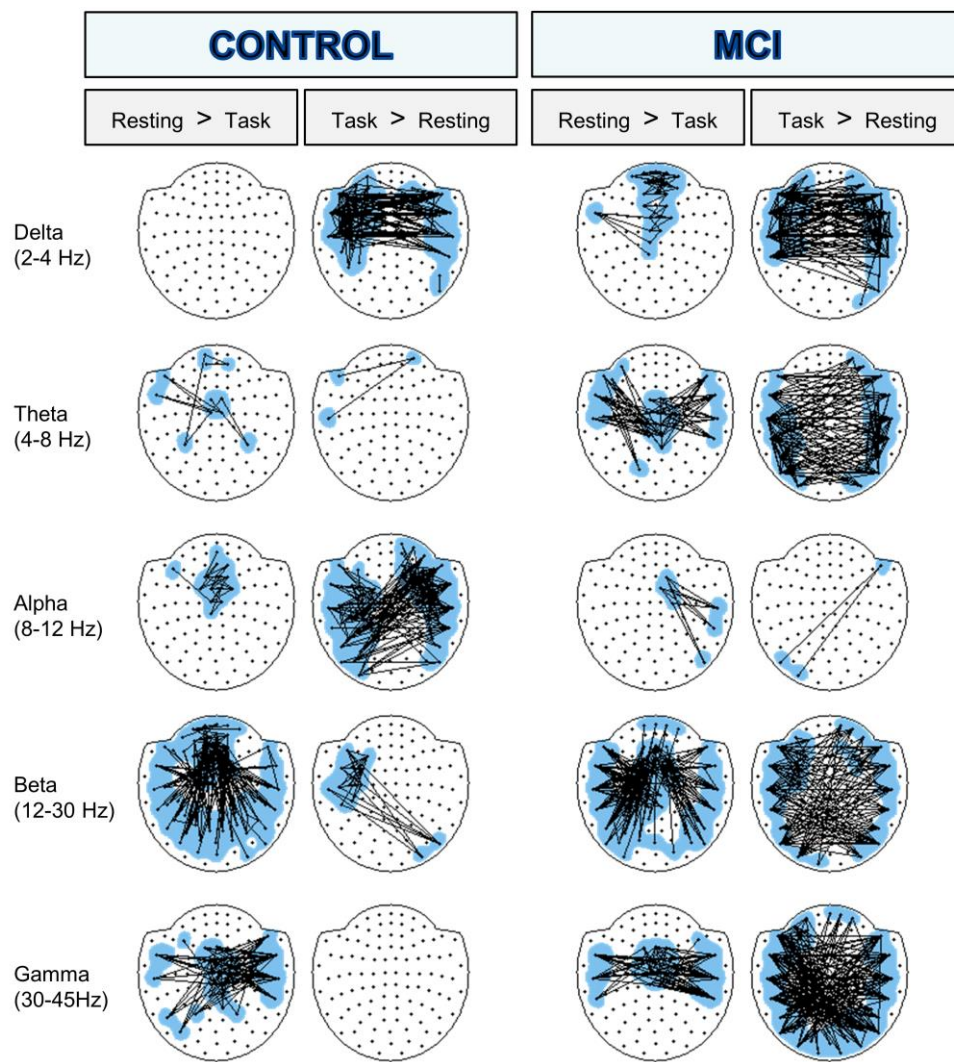
**Table 2.** IDICS performance in Controls and MCIs. Mean values  $\pm$  standard deviation of the responses given by the participants after 1 minute of subtraction. Numbers of the 1-subtraction condition: 75, 99, 158 and 246. Numbers of the 3- subtraction condition: 89, 97, 174 and 237. The p values were obtained with a Mann-Whitney *U* test.

Numbers	Mean values $\pm$ standard deviation		P values
	Controls	MCIs	
<b>75</b>	14,67 $\pm$ 17,20	23,00 $\pm$ 20,06	P= 0.192
<b>99</b>	39,50 $\pm$ 21,48	55,14 $\pm$ 34,81	P= 0.226
<b>158</b>	102,12 $\pm$ 30,04	102,36 $\pm$ 37,96	P= 0.594
<b>246</b>	184,92 $\pm$ 37,34	169,73 $\pm$ 51,33	P= 0.639
<b>89</b>	24,65 $\pm$ 24,24	30,50 $\pm$ 24,69	P= 0.349
<b>97</b>	27,58 $\pm$ 17,78	51,36 $\pm$ 26,37	P= 0.051
<b>174</b>	121,00 $\pm$ 43,89	114,77 $\pm$ 36,54	P= 0.983
<b>237</b>	183,28 $\pm$ 34,85	166,18 $\pm$ 45,46	P= 0.186

### 3.2 MEG connectivity results

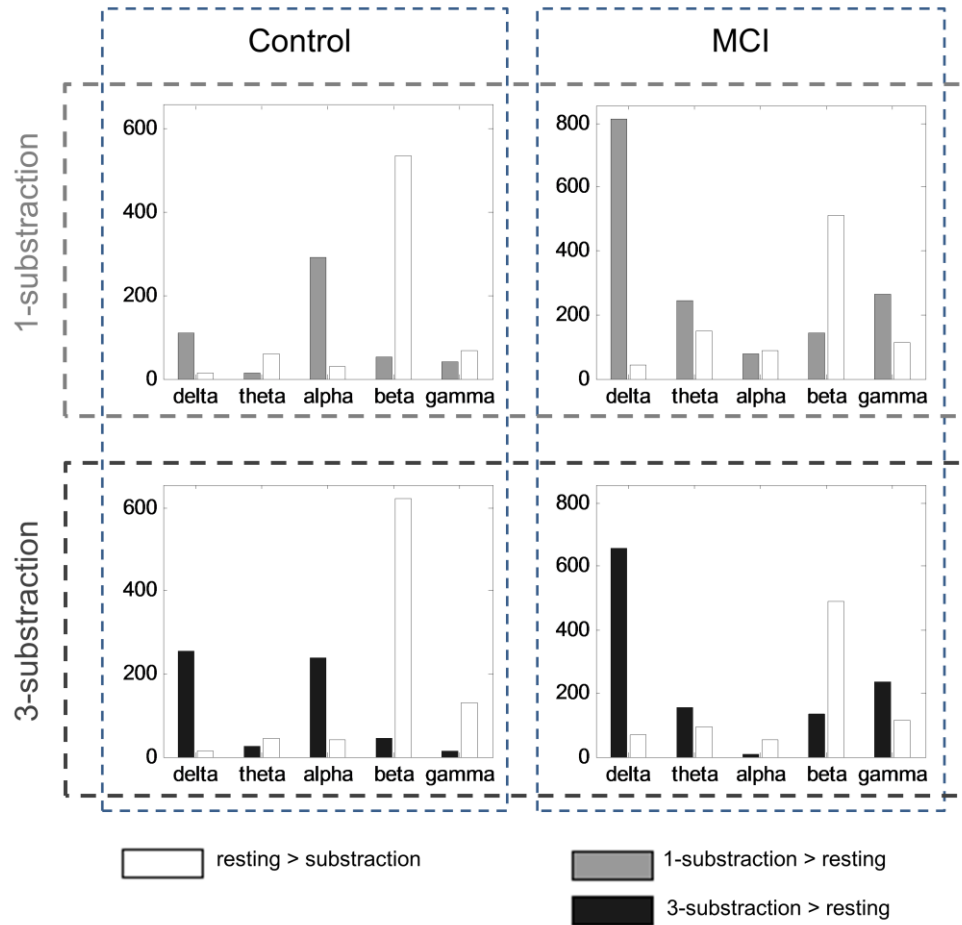
The result of the between-conditions analysis is summarized in Fig. 2 and Fig. 3.

Fig. 2 compares resting state and 3-subtraction task and shows the topography of the modified links. The topography of the connectivity change produced by the 1-subtraction task was not represented for the sake of simplicity. However, in general terms it was similar than the one for the 3-subtraction task.



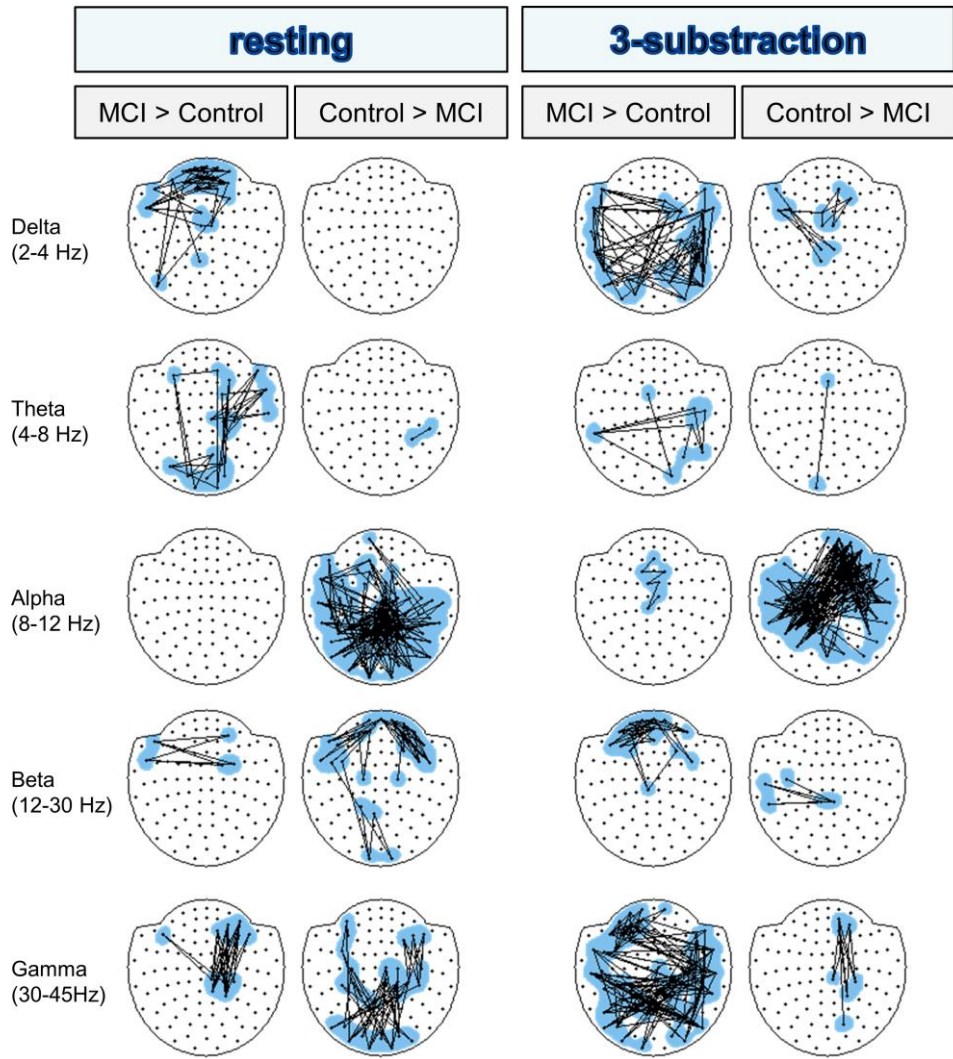
**Figure 2.** Statistical differences in connectivity between resting and 3-subtraction task for controls and MCIs. Significantly altered links ( $p < 0.005$ ) are shown for every frequency band. A line between two nodes represents a link that has been significantly altered, and is either decreased (resting > task) or increased (task > testing) in the 3-subtraction task. For clarity in visualization, only a selection of links was displayed: firstly, in order to reduce loose links, nodes participating with a single significant link were discarded, and secondly, a maximum of 150 links was plotted (if more links were statistically significant, 150 links with the lowest p-value were chosen).

Figure 3 contrasts resting with 1- and 3-subtraction task, displaying the overall amount of altered links and enabling a comparison of the network change produced by both tasks.



**Figure 3.** Number of significantly altered links when comparing resting and task for different groups and frequency bands. The first row shows differences between 1-subtraction task (grey) and resting state (white), the second row considers 3-subtraction (black) and resting. The two groups (controls and MCIs) are represented in different columns. Only links with  $p < 0.005$  were considered.

Finally, Figure 4 represents differences between Controls and MCI for resting state and for a resting-normalized 3-subtraction task separately, allowing a direct contrast between both groups.



**Figure 4.** Statistical differences in connectivity between controls and MCIs in resting state and 3-substraction task. Significantly altered links ( $p < 0.02$ ) are shown for every frequency band. A line between two nodes represents a link that has been significantly altered, and is either increased (MCI > Control) or decreased (Control > MCI) in the MCI group. As in Figure 2, for clarity in visualization, loose links were removed a maximum of 150 links was displayed.

Results will be now described separately for every frequency band, focusing on connectivity differences and neuropsychology correlations.

### 3.2.1 Delta band

During resting state, MCI patients exhibited higher values of connectivity fundamentally over frontal regions than controls (see Fig. 4). This increment was inversely correlated with MMSE ( $r = -0.28$ ,  $p = 0.026$ ); Immediate ( $r = -0.46$ ,  $p < 0.0001$ ) and Delayed Recall ( $r = -0.37$ ,  $p = 0.002$ ); Rule shift Cards ( $r = -0.3$ ,  $p = 0.016$ ); Phonemic ( $r = -0.26$ ,  $p = 0.035$ ) and Semantic Fluency ( $r = -0.35$ ,  $p = 0.003$ ); Boston Naming Test ( $r = -0.41$ ,  $p = 0.001$ ) and directly correlated with TMTA time ( $r = 0.30$ ,  $p = 0.016$ ) and TMTB time ( $r = 0.30$ ,  $p = 0.019$ )

Delta band connectivity pattern was greatly altered by the counting task compared to resting state, showing a big increase of PLV interhemispheric connections in all groups, which were of higher magnitude in MCIs (see Fig. 2 and 4).

The inter-hemispheric increment of synchronization observed in the control group while performing the task was negatively correlated with the Inverse Digit Span Test ( $r = -0.32$ ,  $p = 0.010$ ). Besides, a greater inter-hemispheric synchronization, as MCIs showed, was negatively correlated with hippocampal volume ( $r = -0.27$ ,  $p = 0.049$ ); Inverse digit Span Test ( $r = -0.27$ ,  $p = 0.031$ ); Semantic Fluency ( $r = -0.25$ ,  $p = 0.043$ ) and Immediate ( $r = -0.26$ ,  $p = 0.037$ ) and Delayed Recall ( $r = -0.27$ ,  $p = 0.030$ ), evidencing that a lower behavioral performance is associated with a higher connectivity value. Additionally, a decrease in short range delta connectivity between frontal sensors when performing the task, was positively correlated with TMTA time ( $r = 0.26$ ,  $p = 0.042$ ) and inversely correlated with Semantic Fluency ( $r = -0.25$ ,  $p = 0.043$ ); Immediate ( $r = -0.36$ ,  $p = 0.003$ ) and Delayed Recall ( $r = -0.35$ ,  $p = 0.005$ ) and Boston Naming Test ( $r = -0.31$ ,  $p = 0.014$ ).

### 3.2.2 Theta band

MCIs showed an increase in synchronization over right parieto-temporal areas and between fronto-occipital regions during resting state compared to the control group (see Fig 4). This hyper connectivity was negatively related to the hippocampal volume ( $r = -0.35$ ,  $p = 0.01$ ) and to the scores in MMSE ( $r = -0.30$ ,  $p = 0.015$ ); Clock Drawing test (order subtest) ( $r = -0.39$ ,  $p = 0.001$ ); Immediate ( $r = -0.56$ ;  $p < 0.0001$ ) and Delayed Recall ( $r = -0.55$ ,  $p < 0.0001$ ); Rule shift Cards ( $r = -0.35$ ,  $p = 0.004$ ); Phonemic ( $r = -0.27$ ,  $p = 0.028$ ) and Semantic Fluency ( $r = -0.37$ ,  $p = 0.002$ ); and Boston Naming Test ( $r = -0.34$ ,  $p = 0.002$ ).

Theta band PLV distribution differed greatly in Controls and MCIs during the performance of the task (see Fig 2). In the Control group few differences were found between resting and the mental calculation state, and were not correlated with neuropsychological or hippocampal

measures. However, the MCI group showed both increases and decreases of synchronization during the IDICS execution. The inter-hemispheric desynchronization from central to lateral sensors was inversely correlated with Immediate ( $r = -0.246$ ,  $p = 0.048$ ) and Delayed Recall ( $r = -0.281$ ,  $p = 0.025$ ); and the excess of interhemispheric PLV was directly correlated with TMTA time ( $r = 0.277$ ,  $p = 0.027$ ).

### *3.2.3 Alpha band*

There were clear differences in synchronization in resting state between both groups, since the controls showed higher connectivity values, mainly over central and posterior interhemispheric regions, than the MCI patients (see Fig 4). In addition this increment was positively correlated with several test such as MMSE ( $r = 0.42$ ,  $p = 0.001$ ); Immediate ( $r = 0.40$ ,  $p = 0.001$ ) and Delayed Recall ( $r = 0.44$ ,  $p < 0.0001$ ); Phonemic ( $r = 0.29$ ,  $p = 0.021$ ) and Semantic Fluency ( $r = 0.35$ ,  $p = 0.003$ ); and Boston Naming Test ( $r = 0.30$ ,  $p = 0.016$ ).

In addition, alpha connectivity behaved very differently from the previous bands, and the changes in controls were bigger than in MCIs for this band during the IDICS task (see Fig 2). Controls showed an important increase of interhemispheric PLV and a decrease in short-range coupling in middle frontal sensors during the task, while in the MCI group far fewer links were altered in the transition from the resting to the cognitive state.

The interhemispheric increase in synchronization during the performance of the mental task was directly related to MMSE ( $r = 0.285$ ,  $p = 0.021$ ); Immediate ( $r = 0.372$ ,  $p = 0.002$ ) and Delayed Recall ( $r = 0.309$ ,  $p = 0.013$ ); Phonemic ( $r = 0.294$ ,  $p = 0.018$ ) and Semantic Fluency ( $r = 0.267$ ,  $p = 0.029$ ); Boston Naming Test ( $r = 0.285$ ,  $p = 0.024$ ); TMTA time ( $r = -0.428$ ,  $p = 0.0003$ ) and TMTB time ( $r = -0.369$ ,  $p = 0.003$ ). While the decrease in synchronization in middle frontal regions was positively correlated with hippocampal volume ( $r = 0.35$ ,  $p = 0.011$ ); MMSE ( $r = 0.253$ ,  $p = 0.042$ ); Immediate ( $r = 0.270$ ,  $p = 0.03$ ) and Delayed Recall ( $r = 0.330$ ,  $p = 0.008$ ); Semantic Fluency ( $r = 0.388$ ,  $p = 0.001$ ) and Boston Naming Test ( $r = 0.377$ ,  $p = 0.002$ ) and negatively correlated with TMTA time ( $r = -0.268$ ,  $p = 0.032$ ) and TMTB time ( $r = -0.399$ ,  $p = 0.001$ ). These results points out the important relationship between this frequency band, and the hippocampal volume and the cognitive status.

### 3.2.4 Beta band

Controls showed higher synchronization values over interhemispheric frontal areas and parieto-occipital regions during resting state than the MCI subjects, who exhibited an increase in connectivity between parietal and left temporal areas (see Fig 4). The rise of synchronization over interhemispheric frontal areas and parieto-occipital regions was directly correlated with hippocampal volume ( $r = 0.5$ ,  $p < 0.0001$ ); Immediate ( $r = 0.35$ ,  $p = 0.004$ ) and Delayed Recall ( $r = 0.37$ ,  $p = 0.003$ ) and inversely correlated to TMTA time ( $r = -0.26$ ,  $p = 0.04$ ), while the hypersynchronization between parietal and left temporal areas was negatively related to Clock Drawing test (order subtest) ( $r = -0.37$ ,  $p = 0.002$ ); Immediate ( $r = -0.4$ ,  $p = 0.002$ ) and Delayed Recall ( $r = -0.42$ ,  $p = 0.001$ ); Semantic Fluency ( $r = -0.4$ ,  $p = 0.001$ ); Boston Naming Test ( $r = -0.42$ ,  $p = 0.01$ ); and positively to TMTB time ( $r = 0.31$ ,  $p = 0.015$ ).

During the execution of the task, both groups exhibited an inter-hemispheric and anterior-posterior desynchronization, being higher in the case of the controls, and different patterns of increase of synchronization (see Fig 2). The increase of connectivity observed in the control group between left anterior areas and right posterior regions was not correlated with anatomical and neuropsychological information, while the interhemispheric synchronization found in the MCI group was inversely correlated with Inverse Digit Span ( $r = -0.249$ ,  $p = 0.047$ ) and Immediate Recall ( $r = -0.253$ ,  $p = 0.042$ ).

Although an increase in desynchronization was observed in both groups while performing the task, the pattern of the controls was positively correlated with Clock Drawing Test (order subtest) ( $r = 0.414$ ,  $p = 0.001$ ) and inversely correlated with TMTB time ( $r = -0.280$ ,  $p = 0.029$ ); while the pattern observed in the MCI group was negatively correlated with Inverse Digit Span ( $r = -0.332$ ,  $p = 0.007$ ); and Immediate ( $r = -0.344$ ,  $p = 0.005$ ) and Delayed Recall ( $r = -0.266$ ,  $p = 0.033$ ). Connectivity differences between both groups were mainly located in frontal regions where connectivity was higher in Controls in resting state and higher in MCI during the task, indicating a difficulty for MCIs to desynchronize during the task (see Fig 4).

### 3.2.5 Gamma band

In resting state, controls presented higher connectivity in parieto-occipital sensors than MCIs, while these last showed higher PLV values between right fronto- temporo-parietal areas (see Fig 4). The increase observed in the control group was positively correlated with MMSE ( $r = 0.3$ ,  $p = 0.015$ ); Immediate ( $r = 0.5$ ,  $p < 0.0001$ ) and Delayed Recall ( $r = 0.44$ ,  $p < 0.0001$ ); Semantic Fluency ( $r = 0.4$ ,  $p = 0.001$ ) and hippocampal volume ( $r = 0.5$ ,  $p < 0.0001$ ), whereas the



hypersynchronization between right fronto- temporo-parietal areas was inversely correlated with Clock Drawing Test (order subtest) ( $r = -0.28$ ,  $p = 0.021$ ); Direct Digit Spam ( $r = -0.32$ ,  $p = 0.009$ ); Immediate ( $r = -0.41$ ,  $p = 0.001$ ) and Delayed Recall ( $r = -0.34$ ,  $p = 0.006$ ); Semantic Fluency ( $r = -0.33$ ,  $p = 0.006$ ) and shift Cards ( $r = -0.3$ ,  $p = 0.018$ ).

During the IDICS's execution, both groups presented a central to lateral desynchronization within the gamma band. The desynchronization pattern observed in the control group was directly correlated with the Boston Naming Test ( $r = 0.279$ ,  $p = 0.027$ ); Ideomotor Praxis ( $r = 0.26$ ,  $p = 0.04$ ); and Immediate ( $r = 0.312$ ,  $p = 0.011$ ) and Delayed Recall ( $r = 0.352$ ,  $p = 0.004$ ); while the desynchronization pattern found in the MCI group did not correlate with hippocampal atrophy and cognitive performance.

Only the MCI group showed an increase of inter-hemispheric synchronization that was inversely correlated with Direct ( $r = -0.302$ ,  $p = 0.014$ ) and Inverse Digits Spam ( $r = -0.396$ ,  $p = 0.001$ ) and Immediate ( $r = -0.267$ ,  $p = 0.032$ ); and Delayed Recall ( $r = -0.341$ ,  $p = 0.006$ ). This suggests that hypersynchronization at higher frequencies could be associated with worse cognitive performance in memory tasks.

### *3.3 1-and 3-subtraction tasks*

In general the connectivity patterns in 1- and 3- subtraction tasks were alike. Since topography in both conditions was similar, the overall amount of altered links between resting and 1- and 3- subtraction tasks are shown in Fig. 3, which allows the comparison between the network changes induced by both tasks. In particular, if the transition from resting to 1- and then to 3- subtraction task was linear, one would expect that the differences between resting and task would be much bigger for the 3- than for the 1-subtraction task. And this could be seen through a higher amount of altered links in the 3- than in the 1-subtraction task. For controls, such a tendency was found in delta and beta, and to a lesser extent in the gamma band. On the contrary for MCIs, there was rather a small decrease in the number of links in the 3- subtraction task when compared to the 1-subtraction task.

## 4. DISCUSSION

The aim of this study was twofold: to investigate how functional connectivity changes between resting state and IDICS with increasing processing demands, and to consider how the presence of cognitive impairments in aging modifies these synchronization patterns. To this end we measured MEG signals in healthy elderly adults and in patients diagnosed with MCI, and we employed an internal mental calculation task with two levels of difficulty, 1- subtraction and 3-subtraction. As expected, task load modulated connectivity patterns, which differed between both groups, and MCI displayed hypersynchronization in most frequency bands and a lack of synchronization in the alpha band.

### 4.1 *Resting versus IDICS task*

Delta results are in line with most calculation task studies, which have mainly used EEG and analyzed power distributions (Harmony et al., 1996, 2004; Dimitriadis et al., 2010; Giannitrapani, 1971). Delta band activity is considered to increase during cognitive tasks requiring attention to internal processing and to decrease under conditions which demand attention to the external environment. Additionally, the magnitude of the increment rises with the difficulty of the task (Dimitriadis et al., 2010; Harmony et al., 1996). Here we showed that in both groups delta connectivity was greater during mental calculation than in resting state; a finding in line with the previous literature. This increased connectivity between the left and right anterior regions indicates the necessity of the interaction between both hemispheres for the performance of mental calculation and could be engaged in updating of arithmetic operations and manipulation of information (Krueger, Landgraf, van der Meer, Deshpande, & Hu, 2011).

No important differences in the theta band could be ascertained in the control group. Some studies using EEG to investigate cognition during arithmetic and mental tasks have described an increase in theta-band activity in the frontal midline areas of the brain, although in these cases spectral power was being considered instead of connectivity (Sasaki et al., 1996; Ishii et al., 1999; Onton, Delorme, & Makeig, 2005; Yener et al. 2013). For MCIs, bigger connectivity changes between task and resting were needed to deal with the subtraction task. These results could be explained for two possible reasons: First, individuals in the MCI group found the task more difficult to complete and thus displayed more theta-band activity (theta-band activity being attributed to memory load, complex tasks and attention (Gevins, Smith, McEvoy, & Yu, 1997), and second because the MCI group usually shows an increase in both theta power and

connectivity during the execution of cognitive tasks (Jiang et al, 2005, 2008; Aurtenetxe et al., 2013).

In the alpha band the control group showed an increase in connectivity during the subtraction task. These results are consistent with recent studies which consider that the alpha band is associated with internal tasks (Benedek, Bergner, Könen, Fink, & Neubauer, 2011; Cooper, Croft, Dominey, Burgess, & Gruzelier, 2003; Jensen, Gelfand, Kounios, & Lisman, 2002; S. Palva & Palva, 2007; Sauseng et al., 2005), such as mental calculation, and that may reflect an internal information processing which involves top-down control and may be related to an inhibition of external interfering input (Jensen & Mazaheri, 2010). Controls also displayed a desynchronization over frontal brain areas, which could be related to the processing of semantic information such as mathematical knowledge (Wolfgang Klimesch, Sauseng, & Hanslmayr, 2007). For MCIs, few connectivity changes were found in this band, suggesting that this group had less cognitive control. It could be speculated that the excess synchronization in delta, theta, beta and gamma bands compensated for the lack of connectivity changes in the alpha band.

In the beta band, long distance connections between left anterior region and right posterior areas increased in the control group. Conversely, there was a broad desynchronization in both groups in the IDICS task compared to resting state. This may reflect the activation of areas needed for calculation-such as the fronto-parietal network (Krueger et al., 2011) or the deactivation of others that are able to adapt to the cognitive difficulty. Each factor could play a role in the successful execution of cognitive tasks. These results could be related to the Default Mode Network (DMN) (Raichle et al., 2001), which is an antero-posterior network that deactivates when performing a task. So the DMN might behave in the same way in internal and external cognitive tasks, although further studies would be needed to test this hypothesis. Finally, our results suggest that the main differences from group to group were found in the anterior regions of the DMN. These regions were found to be more desynchronized in the control group when compared to MCIs, indicating that MCIs were not able to sufficiently deactivate this network to perform the task.

Similar to the beta-band findings, a desynchronization of the resting state networks of both groups was noted during task performance when looking at results in the gamma band. In this line, Park et al. (2012) found higher gamma event related desynchronization in elderly controls when compared to a group of MCI patients, which was related to cognitive performance.

#### *4.2 Control group vs MCI group*

As expected, differences in resting state connectivity between Control and MCI were found. The MCI group presented a higher synchronization than the control group in low frequency bands (e.g. frontal delta and antero-posterior right theta) and this increase related to hippocampal atrophy (i.e. theta band) and to a lower global cognitive status (i.e. MMSE) and worse performance in attention, memory, language and executive functioning. On the contrary, they exhibited lower connectivity values in the alpha band, especially inter-hemispheric and in central and posterior regions, as well as the beta band in frontal areas. The desynchronization observed in the MCI group was related to poorer performance in multiple cognitive domains, such as memory or executive function, and also with a smaller hippocampal volume. Finally, MCIs showed a higher synchronization in the gamma band between right anterior and central areas, which was related to a poorer performance in attention, memory and executive function, while in the control group this increase was localized in more posterior regions and was directly correlated with a greater hippocampal volume and higher scores in MMSE, Immediate and Delayed Recall and Semantic Fluency. Most of these findings, and especially the decrease in synchronization in alpha and beta bands, are in agreement with those found in MCI and AD studies (Berendse et al. 2000; Jelic et al. 2000; Koenig et al. 2005; Stam, van der Made, Pijnenburg, & Scheltens, 2003; Moretti et al., 2008; Gómez 2009), suggesting that MCI could be considered as the beginning of the “disconnection syndrome” (Delbeuck et al., 2003).

For the subtraction task MCIs showed an increase of synchronization in most frequency bands during the execution of the task when compared to the resting state. This points out that the MCI were calculating in a rather inefficient way, since they needed more brain connections than the control group to perform the same task. These findings are in agreement with many studies which employed cognitive tasks in MCI population (Bajo et al., 2010; Jiang & Zheng, 2006; Jiang, 2005; Pijnenburg et al., 2004). In the alpha band however, MCIs were desynchronized during the task. This could mean that MCI showed an impairment in the mechanisms related to top-down control and inhibition of external inputs and (Wolfgang Klimesch et al., 2007), which would indicate that MCIs struggled with centering on the task. On the whole, these synchronization/desynchronization profiles could be used as a potential biomarker of the MCI disease.

To further investigate the meaning of the hyper/hyposynchronization found during the execution of the IDICS task and their relation to brain atrophy and cognitive behavior, connectivity changes were correlated with hippocampal volumes and neuropsychological tests. In the MCI group, the increases and decreases found in synchronization in delta, theta, beta and gamma bands during the execution of the task correlated with a lower performance in most of the neuropsychological measures employed, including semantic and episodic memory, attention and executive functioning. Additionally, higher PLV values in delta band during IDICS were related to lower hippocampal volume, indicating that delta hypersynchronization during the execution of the task relates to anatomical and functional deterioration. It is noteworthy that in the control group, an interhemispheric increase in the alpha band was directly correlated with global cognitive status (i.e. MMSE), semantic and episodic memory, and executive functioning, while a frontal decrease in connectivity was also related to the hippocampal volume. These results indicate that the ability to modulate the alpha band is essential for a good cognitive functioning.

#### *4.3 Task load*

In delta, beta and to a lesser extent in gamma frequency bands, the number of altered links was higher in the 3- than the 1-subtraction task when compared against the resting state, indicating that task load modulates connectivity changes. Other studies have described how brain activity increases with task difficulty or cognitive load in ageing (Morcom, Li, & Rugg, 2007; Rypma, Eldreth, & Rebbelchi, 2007; Zarahn, Rakitin, Abela, Flynn, & Stern, 2007). Palva et al. (2010) found that an increase working memory load resulted in a strengthened brain synchrony. Micheloyannis et al. (2005) suggested that increasing the complexity of arithmetic tasks by increasing the demand for additional operations is associated with a more widespread synchronization pattern. However, this increase of altered connections with task difficulty was not present in MCI. This effect could be interpreted under the framework of the CRUNCH model (Compensation-Related Utilization of Neural Circuits Hypothesis (Reuter-Lorenz & Cappell, 2008), which was introduced to explain how cortical circuits adapt to increasing task load. When the old and young subjects are compared, the older cohort recruit more neural resources to acquire a similar performance at low levels of task demand, leading to higher levels of neural activity. Regardless, they have fewer resources available to meet the processing requirements of more demanding task (as a resource ceiling is reached) and their performance then declines. A similar effect seems to occur in this study, when elders with and without cognitive impairment were compared. In the control group, the synchronization

changes rose with the difficulty of the task (i.e. Delta band), while in the MCI group more resources were needed to achieve the same level of performance in the 1-subtraction task, but a decline in synchronization changes was noted in the 3-subtraction condition. However, further studies with increasing load are needed to verify this effect in both normal and pathological aging.

In summary, controls exhibited connectivity changes in all frequency bands to deal with the IDICS. These changes were related to the task difficulty, especially in delta and beta frequency bands, according to the CRUNCH model. MCIs showed different connectivity profiles than controls, pointing out that the MEG could contribute to the assessment of the MCI diagnosis, in addition to that of the biomarkers commonly used in clinical practice (Molinuevo & Rami, 2013). MCIs showed a higher synchronization increase to perform the task, except from the alpha band. This increment did not correlate positively with the task load, and was associated with a lower behavioral cognitive function. In fact, this hyperconnectivity in MCI has been found previously in working memory tasks with external stimuli (Bajo et al, 2010), and was used as a marker for prediction of conversion to dementia (Bajo et al. 2012). It can be speculated that the profile of hypersynchronization found here in the MCI patients may be related to the pathological process of AD. This view of synchronization as pathological, not as compensatory, would be in agreement with the hypothesis proposed by de Haan et al. 2012). They postulated that the increase in connectivity reported in MCIs might be due to higher amyloid accumulation over the most connected regions (hubs), which are especially vulnerable to AD.. In this line, hyperactivity has been associated with the release of beta amyloid protein into interstitial fluid and neuronal hyperexcitability (Cirrito et al., 2008). In fact, recent findings indicate that in the vicinity of the beta amyloid plaque there is a decrease in the number of gabergic neurons (Garcia-Marin et al., 2009) which could increase the hyperexcitability. Thus, the greater the excitability, the greater the likelihood of neuronal synchronization and as a consequence a profile of widespread connectivity as is the case of the MCI patients.

The internal cognitive task introduced here differs from the most commonly employed cognitive paradigms, because of the absence of external stimuli, which requires inhibition from both the environment and internal thoughts. This would suggest that the MCIs had difficulties with top-down control processes during the calculation task, since they failed to synchronize in alpha band. Impairment in inhibitory control mechanisms may affect the performance of daily living activities, contributing to the patient's progressive inability to adapt to the environment and to control for internal emotional stimuli (Fujie et al., 2008). Thus, the IDICS allows a better comparison with the resting state condition than usual external tasks, as it resembles the

resting condition more, and provides relevant information regarding the brain network impairment in MCI.

#### *4.4 Limitations of the study*

It is important to note that this study presents some limitations. First of all, the IDICS task employed here is advantageous and novel in that it enables the study of brain alterations in MCI independently from the effect of external stimuli. However, it has a clear drawback: as an internally-driven task, its performance is difficult to assess externally. The consideration of the final number that was reached from the subjects after a subtraction exercise provides some insight into the task execution, but it is not a direct metric of performance. Second, we analyzed functional connectivity in classically defined frequency bands, with fixed frequency limits: delta (2-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), gamma (30-45 Hz). However, we did not examine power spectra. A future spectral analysis could provide insight on how brain rhythms change with the IDICs task and with the MCI disease. Third, the analysis was performed in sensor-space and therefore lacks spatial accuracy. Future studies in source space could locate the brain areas and the networks, as the DMN, involved in the functional connectivity changes in MCI. Fourth, as the calculation task used requires different cognitive processes, factors such as attention could be affecting the task execution. Fifth, to propose the MEG as a new potential biomarker it should be compared with the established biomarkers in dementia research such as cerebrospinal fluid (CSF) and Positron emission tomography with Pittsburgh compound B (PET-PIB) (Dubois et al., 2010).

## **STUDY III**

**Hypersynchronization in alpha band differs progressive  
from stable mild cognitive impairment. A MEG study**



## **1. AIMS AND HYPOTHESIS**

As there is a lack of studies based on a non-invasive technique which: 1) explore the brain regions which are affected in pMCI from a synchronization framework (previous functional connectivity studies with EEG/MEG were done in the sensor space); and 2) combine information of brain damage (i.e. MR-volumetry), functional network organization and cognitive status, the present predictive resting state MEG study in source space would integrate neuropsychological, neurophysiological and structural information in order to compare sMCI and pMCI patients. According to previous neurophysiological studies, we predict that pMCI patients will show a higher synchronization in high frequency bands primarily over posterior brain areas compared to sMCI participants.

## 2. METHODS

### 2.1 Subjects

For the present study 49 MCI patients were recruited from the Neurology and Geriatric and departments of the “Hospital Universitario San Carlos, Madrid” and the “Memory Decline Prevention Centre, Madrid”. All of them were right handed (Oldfield, 1971) and native Spanish speakers. All participants were monitored yearly over 2 year follow- up period, with a clinical examination every six months. According to their clinical follow-up, they were divided in two subgroups: stable MCI patients (n=30) and progressive MCI patients (n=19). The pMCI subjects used in this study can be considered faster converters ( $13.16 \pm 5.87$  (mean  $\pm$  SD) months) (Okello et al., 2009).

Both groups were homogenized in age (mean age sMCI: 74.03 years; pMCI: 76.68 years;  $p = 0.104$ ), gender (males/females sMCI: 14/16; pMCI: 8/11;  $p = 0.777$ ) and educational level (sMCI: 2.79; pMCI: 2.68;  $p = 0.934$ ).

### 2.2 Diagnostic criteria

The Mini Mental State Examination (MMSE; Lobo et al., 1979), the Geriatric Depression Scale (Yesavage et al., 1982) the Global Deterioration Scale (GDS; Reisberg et al., 1982), the Hachinski Ischemic Score (HIS; Rosen et al., 1980), the Functional assessment questionnaire (FAQ; Pfeffer et al., 1982), the questionnaire for Instrumental Activities of Daily Living (IADL; Lawton & Brody, 1969), and the Functional Assessment Staging (GDS/FAST; Auer & Reisberg, 1997) were used to establish the global cognitive and functional status of the patients.

MCI diagnosis was established according to Petersen’s criteria (R C Petersen, 2004): (1) memory complaint confirmed by an informant; (2) abnormal memory function; (3) normal general cognitive function; (4) total absence or minimal impairment in activities of daily living (ADLs); and (5) not sufficiently impaired to fulfil the dementia’s criteria by the clinical guidelines of the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) (G. McKhann et al., 1984). In addition, all subjects underwent an extensive neuropsychological assessment to evaluate their cognitive deficits in multiple areas with the following tests: Clock Drawing Test (Agrell & Dehlin, 1998), Direct and Inverse digit Span Test (Wechsler Memory Scale Revised, WMS-III; Wechsler, 1987), Immediate and Delayed Recall (WMS-III; Wechsler, 1987), Phonemic and Semantic Fluency (Controlled Oral Word Association Test, COWAT, Benton & Hamsher, 1989),

Ideomotor Praxis of Barcelona Test (Peña-Casanova, 1990), Rule shift Cards (Behavioural Assessment of the Dysexecutive Syndrome, BADS; Norris & Tate, 2000), Visual Object and Space Perception Test (VOSP; Warrington & James, 1991), Boston Naming Test (BNT; Kaplan et al., 1983) and Trail Making Test A and B (TMTA and TMTB; Reitan, 1958).

All of them were classified as amnesic MCI patients. All subjects were re-evaluated every 6 months in order to assess their evolution. Therefore, those patients who finally fulfilled the criteria for probable AD according to the guidelines of the NINCDS-ADRDA (G. McKhann et al., 1984) were classified as pMCI.

The whole sample of MCI patients was free of significant neurological or psychiatric diseases (other than AD or MCI) and medical treatment which could affect MEG activity (e.g. cholinesterase inhibitors). General inclusion criteria considered an age between 65 and 85 years, a modified Hachinski score less than or equal to 4, a Hamilton Depression Rating Scale score less than or equal to 9 and a magnetic resonance imaging (MRI) within 12 months before MEG screening without indication of infection, infarction, or focal lesions.

The study was approved by the local ethics committee and, before the MEG recordings; a written informed consent was signed from all subjects or their legal representatives.

### *2.3 Hippocampal volumes*

Hippocampal volumes were measured as anatomical evidences of brain atrophy characteristic for MCI and AD (Dubois et al., 2007). In addition, this measure has previously shown its predictive power in AD progression (Clifford R Jack et al., 1999). For most of the subjects included in this paper (23 sMCI and 14 pMCI) a T1- weighted MRI was available, acquired in a General Electric 1.5 Tesla magnetic resonance scanner, using a high-resolution antenna and a homogenization PURE filter (Fast Spoiled Gradient Echo sequence, TR/TE/TI = 11.2/4.2/450 ms; flip angle 12°; 1 mm slice thickness, 256x256 matrix and FOV 25 cm). These MRI images were processed with Freesurfer software (version 5.1.0) and its specialized tool for automated cortical and subcortical segmentation (Fischl et al., 2002) in order to obtain the volume of several brain areas. Finally, hippocampal volume was normalized with respect to the overall intracranial volume (ICV) to account for differences in head volume over subjects.

## *2.4 MEG recordings*

Neurophysiological data was acquired by using a 306 channel (102 magnetometers, 204 planar gradiometers) Vectorview MEG system (Elekta AB, Stockholm, Sweden), placed in a magnetically shielded room (VacuumSchmelze GmbH, Hanua, Germany) at the “Laboratory of Cognitive and Computational Neuroscience” (Madrid, Spain). All recordings were obtained in the morning, while subjects were comfortably resting with eyes closed, but awake. Three minutes of MEG signal was acquired for each subject.

Head shape was obtained by using a three-dimensional Fastrak digitizer (Polhemus, Colchester, Vermont) by acquiring three fiducial points (nasion and left and right preauricular points) and at least 300 points of the surface of the scalp. In addition, four head position indication (HPI) coils were placed in the subjects head, two in the mastoids and two in the forehead. HPI coils' position was also acquired using the Fastrak device, in order to provide continuous head position estimation during the recording. Finally, a vertical electrooculogram was placed near the left eye of the subjects to capture the blinks and eye movements.

MEG data was acquired using a sampling rate of 1000 Hz and an online anti-alias filter between 0.1 and 330 Hz. Recordings were offline filtered using a tempo-spatial filtering algorithm (tSSS, correlation window 0.9, time window 10 seconds) (S Taulu & Simola, 2006; Samu Taulu & Kajola, 2005) to subtract the sources of noise placed outside the head, and the head movements were corrected using the same algorithm.

The signal coming from the planar gradiometers was discarded, and only magnetometers were used in the subsequent analysis. Ocular, muscular and jump artefacts were identified using an automatic procedure from the Fieldtrip package (Oostenveld et al., 2011), and confirmed by a MEG expert. The remaining data was segmented in four seconds epochs of artefact-free activity. Only subjects with at least 15 segments were selected for further analysis ( $27.23 \pm 5.99$  epochs in the sMCI group,  $26.11 \pm 6.73$  epochs in the pMCI group, mean  $\pm$  standard deviation). In addition, an ICA-based procedure was employed to remove the electrocardiographic artefact when it was easily identified.

Artefact-free epochs were filtered in six bands: delta (2 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 12 Hz), beta1 (12 to 20 Hz), beta2 (20 to 30 Hz) and gamma (30 to 45 Hz), using a 1500 order FIR filter with Hamming window. The segments were padded with 1.75 seconds of real signal from both sides (1750 samples) to prevent edge effects inside the data.

### *2.5 Source reconstruction*

A source reconstruction analysis was performed independently for each band, using Linearly Constrained Minimum Variance (LCMV) beamformer (Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997). As we did not have a T1 MRI for all subjects a 1mm resolution template of healthy adults (52 subjects, ages  $69.9 \pm 4.4$ , mean  $\pm$  standard deviation) normalized to the Montreal Neurological Institute (MNI) 1mm voxel size template was used to place the sources inside the brain. T1-weighted images were co-registered to the MNI T1 template (available in SPM5 <http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>) using linear affine registration with normalized mutual information as fitness function (Collignon & Maes, 1995). The registered images were normalized to the MNI template using a non-linear registration algorithm (Ashburner, 2007) and then smoothed with a Gaussian kernel with full width half maximum (FWHM) of 4 mm. The resulting normalized images were averaged across subjects to obtain the T1-weighted template. This template was used to define a homogeneous grid of 1cm, and then both template and grid were linearly transformed to fit the head shape of each subject.

The leadfields were defined using a local spheres approach to fit the head shape of each subject in the vicinity of each sensor. Spatial filter coefficients were estimated for each subject using the computed leadfield and an average of the covariance matrix for all the segments. Thereafter, this filter was used to compute the source time series separately for each segment and source location.

Sources were grouped according to the 96 areas Harvard-Oxford cortical map, assigning each source location to the most probable area given its position. Only 1681 source locations labelled as belonging to an area defined in the atlas were considered in the following steps.

### *2.6 Connectivity analysis*

The analysis of the connectivity was performed using the hypothesis of phase synchronization (Rosenblum, Pikovsky, & Kurths, 2001) evaluated by means of the Phase Locking Value (PLV) (Lachaux, Rodriguez, Martinerie, & Varela, 1999). Phase synchronization measures found in the hypothesis that the difference of phases between two phase-locked systems must be non-uniform, and so the degree of non-uniformity must be a good estimator of the coupling level.

In PLV the degree of non-uniformity is calculated by defining as many vectors as temporal points, each one with unity norm and phase equal to the difference of phases of both systems for this temporal point. The norm of the mean vector is the PLV value, ranging from 0, when the differences of phases are uniformly distributed, to values near to 1, when the phases are

concentrated in a small portion of the available range. The formal definition for the PLV value between two systems,  $i$  and  $j$ , with instantaneous phases  $\varphi_i(t)$  and  $\varphi_j(t)$ , is (Lachaux et al., 1999):

$$PLV_{i,j} = \left| \frac{1}{T} \sum_t e^{2\pi j(\varphi_i(t) - \varphi_j(t))} \right|$$

To reduce the dimensionality of the information, we estimated the synchronization between each pair of areas of the Harvard-Oxford cortical atlas. We can consider each area as a system, and each source position inside the area as a noise-contaminated realization of the system. For each pair of areas, area  $i$  with  $M_i$  sources and area  $j$  with  $M_j$  sources, we can create  $M_i$  by  $M_j$  combinations of sources for each segment, leading to  $M_i$  by  $M_j$  repetitions of the experiment. All this repetitions are time locked, thus the total synchronization can be obtained as the norm of the mean vector for all the repetitions.

On the other hand, different segments are not time-locked, as in resting state there is no triggering event that resets the phases (Lachaux et al., 1999). We can then average the synchronization value of all the  $K$  segments for each pair of areas, obtaining a consistent indicator of the level of coupling between the areas. Formally, this PLV index between the areas  $i$  and  $j$  is calculated by:

$$PLV_{i,j} = \frac{1}{K} \sum_K \left| \frac{1}{M_i \cdot M_j \cdot T} \sum_{M_i} \sum_{M_j} \sum_T e^{2\pi j(\varphi_{m_i}(t,k) - \varphi_{m_j}(t,k))} \right|$$

where  $\varphi_{m_i}(t,k)$  represents the instantaneous phase the time series  $i$  of the area  $m$  for the instant  $t$  of the segment  $k$ .

## 2.7 Statistical Analysis

To assess the significant differences in functional connectivity measures we performed a Mann-Whitney  $U$  non-parametrical test using the PLV values estimated for each pair of areas. To correct the multiple comparisons problem we performed firstly a non-parametric permutation tests, keeping the size of the groups but randomly permuting their members (10,000 permutations). The  $p$ -value obtained in this step represents the portion of permutations in which the  $U$  statistic was greater than that in the original data set.

Then a False Discovery Rate (Benjamini & Hochberg, 1995) with  $q=0.1$  (a 10% false positive probability) was used to reduce the portion of false positives in the obtained  $p$ -value distribution. Only the links that survived the FDR are reported in *Results* section. In addition, a Spearman correlated test was performed between connectivity values in these links and neuropsychological and structural data, in order to better understand its significance.

Finally, a classification analysis was performed using the variables that showed significant differences between both groups by using a linear discriminant analysis with leave-one-out cross validation procedure. Results are described in terms of accuracy, sensibility and specificity. These three parameters represented the total fraction of subjects, the fraction of pMCI patients and the fraction of sMCI patients correctly classified, respectively.

### 3. RESULTS

As the diagnosis criteria for AD according to the NINCDS-ADRDA is based on neuropsychological performance, our first approach was to evaluate the predictive capacity of these tests to discriminate between sMCI and pMCI. Table 1 shows the statistical values associated with each cognitive measurement. MMSE scores showed no differences between groups. In contrast, both groups of patients did differ in Immediate ( $p < 10^{-5}$ ) and Delayed Recall ( $p < 10^{-4}$ ), Rule shift Cards ( $p < 0.05$ ) and Semantic Fluency ( $p < 0.05$ ). A classification analysis was performed for each one of the two most significant, achieving classification rates of 77.6% for Immediate Recall (70.0% sensibility, 89.5% specificity) and 66.7% for Delayed Recall (50.0% sensibility, 94.4% specificity). On the other hand, hippocampal volumes, a common indicator of progression in previous studies, showed no differences between groups.

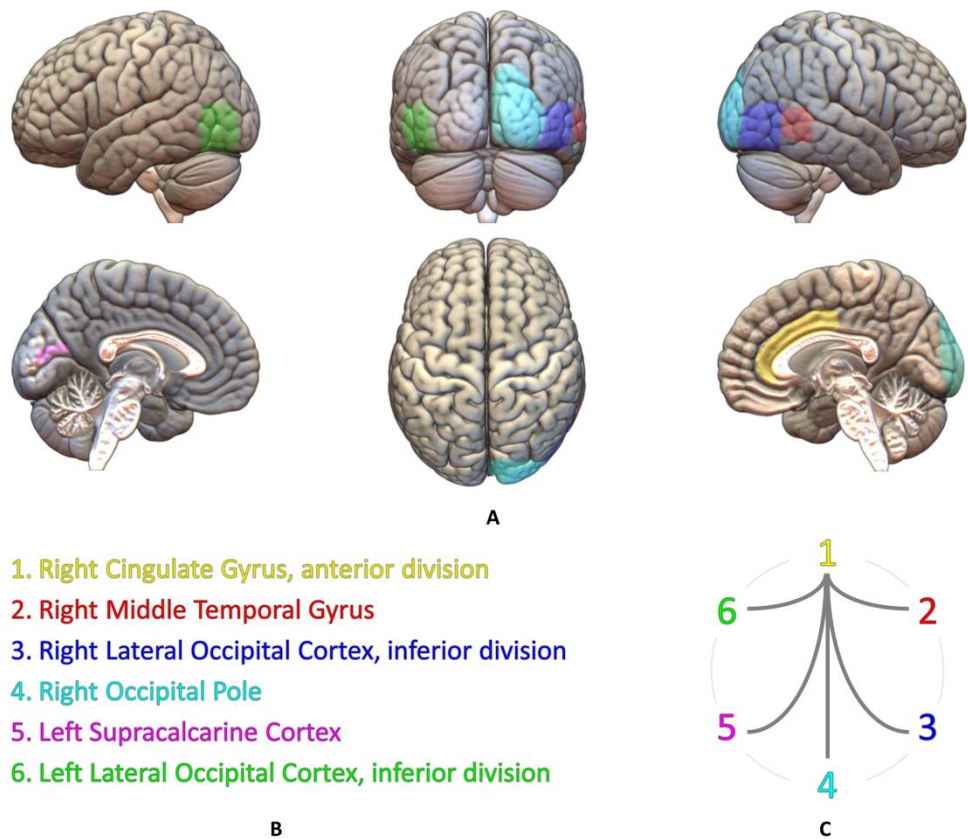
**Table 1.** Demographic, anatomical and neuropsychological information. Mean values  $\pm$  standard deviation of the demographic and clinical characteristics of the Stable MCI patients (SMCI) and Progressive MCI patients (PMCI). M = males; F = females; MMSE = Mini Mental State Examination; GDS= Global Deterioration Scale; FAQ= Functional Activity Questionnaire; GDS- 15= Geriatric Depression Scale; BNT= Boston Naming Test; VOSP= Visual Object and Space Perception Battery; TMTA (acc.) = Trail Making Test part A (accuracy); TMT B (acc.) = Trail Making Test part B (accuracy). Educational level was grouped into five levels: 1: illiterate, 2: primary studies, 3: Elemental studies, 4: High school studies, 5: University studies. P-values for between-groups differences were introduced and significant values ( $p < 0.05$ ) were marked with an asterisk (\*). Wilcoxon-Mann-Whitney test was used for continuous variables (age, educational level, MMSE, Immediate and Delayed recall, Rule Shift Cards, Semantic Fluency, and left and right normalized hippocampus volumes) and Fisher's exact test for gender differences.

	Stable MCI patients (SMCI) (n=30)	Progressive MCI patients (PMCI) (n=19)	P- values
Age (years)	74.03 $\pm$ 5.34	76.68 $\pm$ 5.282	0.104
Gender (M/F)	14/16	8/11	0.777
Educational level	2.79 $\pm$ 1.26	2.68 $\pm$ 1.00	0.934
MMSE score	27.9 $\pm$ 1.86	27.40 $\pm$ 1.96	0.455
GDS	3 $\pm$ 0	3 $\pm$ 0	1
FAQ	1.91 $\pm$ 1.78	1.82 $\pm$ 2.75	0.501



<b>GDS-15</b>	3.83 ± 3.08	3.5 ± 3.82	0.647
<b>Immediate Recall</b>	20.7 ± 8.17	10 ± 4.98	3.26 x 10 <sup>-6</sup> *
<b>Delayed Recall</b>	8.63±7.39	1.78 ± 2.21	7.18 x 10 <sup>-5</sup> *
<b>Rule shift Cards</b>	2.41±1.26	1.56 ± 1.19	0.028*
<b>Semantic Fluency</b>	13±2.81	11.579 ± 4.36	0.041*
<b>BNT</b>	50.41 ± 10.69	46.32 ± 9.36	0.510
<b>Clock Drawing Test (copy)</b>	7.64 ± 2.64	8 ± 4.17	0.60
<b>Clock Drawing Test (order)</b>	6.41 ± 2.62	7.39 ± 3.85	0.615
<b>Direct digit Spam</b>	6.73 ± 1.63	6.37 ± 1.46	0.413
<b>Inverse digit Spam</b>	4.67 ± 1.26	4 ± 1.37	0.103
<b>VOSP</b>	6.68 ± 3.45	7.16 ± 3.06	0.593
<b>Phonemic Fluency</b>	10.837 ± 4.22	8.789 ± 4.08	0.119
<b>TMT A (time)</b>	78.7 ± 36.03	82.83 ± 33.64	0.58
<b>TMT A (acc.)</b>	24 ± 0.58	23.83 ± 1.79	0.401
<b>TMT B (time)</b>	223.17 ± 107.75	258.38 ± 121.71	0.329
<b>TMT B (acc.)</b>	20.1±4.84	15.21 ± 8.60	0.50
<b>Ideomotor Praxis</b>	7.25 ± 1.73	7.21 ± 1.13	0.374
<b>Left hippocampus</b>	0.002217 ± 0.0005075	0.001992 ± 0.0002537	0.056
<b>Right hippocampus</b>	0.002197 ± 0.0004656	0.001965 ± 0.0003882	0.063

The functional connectivity analysis exhibited no significant differences between groups after the FDR in delta, theta, beta1, beta2 or gamma bands. In contrast, FDR analysis in Alpha band showed five significant links between the Right Anterior Cingulate Gyrus and Right Middle Temporal Gyrus and Occipital regions in both hemispheres. In all these links pMCI patients showed significantly greater connectivity values than sMCI patients ( $p < 10^{-3}$ ). Figure 1 depicts the position and size of all six regions implied in these links, and Table 2 shows the  $p$ -value of each one. Afterwards, a classification analysis was performed individually for each link, achieving a maximal classification rate in Link 4 (Right Lateral Occipital Cortex, inferior division with Right Cingulate Gyrus, anterior division) with an accuracy of 81.6% (83.3% sensibility, 78.9% specificity). All classification statistics are showed in Table 2.



**Figure 1.** Cortical areas implied in the five connectivity links that showed significant differences between groups. **A:** Different views of the brain based in MNI template and Harvard-Oxford cortical areas involved in those links. **B:** Relation of areas, numbers and colours. **C:** Significant connectivity links established between those areas.

**Table 2.** The five significant links in the alpha band between sMCI patients and pMCI were obtained with a Mann-Whitney *U* test after the FDR. **Link 1:** *Right Cingulate Gyrus, anterior division with Right Occipital Pole.* **Link 2:** *Right Cingulate Gyrus, anterior division with Right Middle Temporal Gyrus, temporo-occipital part.* **Link 3:** *Right Cingulate Gyrus, anterior division with Left Lateral Occipital Cortex, inferior division.* **Link 4:** *Right Cingulate Gyrus, anterior division with Right Lateral Occipital Cortex, inferior division.* **Link 5:** *Right Cingulate Gyrus, anterior division with Left Supracalcarine Cortex.* **ACC:** Accuracy. **SEN:** Sensibility. **SPE:** Specificity.

	Stable MCI patients (sMCI) (n=30)	Progressive MCI patients (pMCI) (n=19)	<i>p</i> - values	Classification statistics  ACC (SEN, SPE)
<b>Link 1</b>	0.095 ± 0.02	0.132 ± 0.03	1.73 x 10 <sup>-4</sup>	77.6% (83.3%, 68.4%)
<b>Link 2</b>	0.105 ± 0.03	0.140 ± 0.02	2.04 x 10 <sup>-4</sup>	77.6% (76.7%, 78.9%)
<b>Link 3</b>	0.101 ± 0.031	0.141 ± 0.03	2.81 x 10 <sup>-4</sup>	69.4% (70.0%, 68.4%)
<b>Link 4</b>	0.100 ± 0.03	0.142 ± 0.03	3.40 x 10 <sup>-5</sup>	81.6% (83.3%, 78.9%)
<b>Link 5</b>	0.141 ± 0.03	0.182 ± 0.03	1.35 x 10 <sup>-4</sup>	77.6% (76.7%, 78.9%)

In order to better describe our functional connectivity findings we performed a Spearman correlation test between connectivity values in those links, neuropsychological punctuations and volumetric data. We found an inverse significant correlation ( $p < 0.05$ ) between some of this links and MMSE, Immediate Recall and Semantic Fluency scores. Moreover, we observed inverse significant correlations ( $p < 0.05$ ) between several links and both hippocampus volumes. Table 3 details all these findings along with their significance level.

**Table 3.** Spearman correlation analyses of the five significant links obtained in the alpha band with neuropsychological test scores and hippocampal volumes in the whole sample. The table shows the “r” (correlation index) and the “p” values that were significant ( $p < 0.05$ ). MMSE= Mini Mental State Examination.

	Link 1	Link 2	Link 3	Link 4	Link 5
<b>MMSE</b>	r= -0.207 n.s.	r= -0.195 n.s.	r= -0.097 n.s.	r= -0.300 p= 0.047	r= -0.143 n.s.
<b>Immediate Recall</b>	r= -0.366 p= 0.010	r= -0.268 n.s.	r= -0.350 p= 0.014	r= -0.394 p= 0.005	r= -0.300 p= 0.036
<b>Semantic Fluency</b>	r= -0.264 n.s.	r= -0.172 n.s.	r= -0.135 n.s.	r= -0.286 p= 0.047	r= -0.145 n.s.
<b>Right hippocampus</b>	r= -0.342 p= 0.033	r= -0.223 n.s.	r= -0.431 p= 0.006	r= -0.302 n.s.	r= -0.368 p=0.021
<b>Left hippocampus</b>	r= -0.170 n.s.	r= -0.322 p= 0.46	r= -0.213 n.s.	r= -0.370 p=0.20	r= -0.336 p=0.036

Finally, with the aim of evaluating the combined predictive power of both neuropsychological and neurophysiological data in terms of conversion, we performed a classification analysis using as variables those with higher classification statistics: Immediate Recall scores and Link 4 connectivity values. The accuracy achieved combining both measures was 83.7% (80.0% sensibility, 89.5% specificity), greater than if these two variables were considered separately.

## DISCUSSION

This study provides a specific pattern of functional network organization in resting state in those MCI patients who developed dementia. pMCIs showed an increase in functional connectivity values in the Alpha band between anterior and posterior brain areas, and more specifically between right anterior cingulate and temporo-occipital areas, mainly of the right hemisphere.

These results are in agreement on one hand with those obtained by Rossini et al. (2006), since pMCI patients exhibited an increase in fronto-parietal coherence of the right hemisphere in several frequency bands when comparing to sMCIs. And on the other hand, with those described by Bajo et al. (2012), as they found an increased synchronization over temporo-posterior sensors in alpha and beta 1 bands in pMCI subjects compared to sMCIs. Although the increase in synchronization observed in MCIs has been usually interpreted as a compensatory mechanism, the negative correlations found in this study with neuropsychological performance and brain volume could lead us to consider two possible hypotheses about this hypersynchronization: 1) it could be an unsuccessful compensation: the right anterior cingulate along with temporal and occipital regions try to assume the functions of other brain circuits which are more impaired (i.e. involving the posterior cingulate), but in an inefficient way, or 2) it could be the result of an inhibitory deficit: the loss of gabaergic synapses caused by the accumulation of AB plaques (Garcia-Marin et al., 2009) leads it to establish aberrant relationships between areas which start to be affected as the disease progresses.

Anterior cingulate is a region of the medial-frontal circuit that has been related to conflict monitoring, decision making and affective behaviour, among other functions (Devinsky, Morrell, & Vogt, 1995). It has been found a relationship between its alteration in AD patients and inhibition disabilities, unawareness of deficits and apathy (Amanzio et al., 2011; Mori et al., 2013). The executive and inhibitory deficits found even in pure amnesic MCI patients may be also due to the damage of this brain structure (Johns et al., 2012; Kramer et al., 2009). In addition some MRI, SPECT and PIB-PET studies pointed out that this densely connected region might be a key during the evolution of AD. Peters et al. (2014) found that one of the best predictors of conversion from MCI to AD after 2 years was the cortical thinning in the right anterior cingulate. Johnson et al. (2007) reported an increased perfusion in pMCIs than sMCIs within 5 years follow up period in rostral anterior cingulate. Recently Okello et al. (Okello et al., 2009) found that faster converters (average of 1 year, as our patients) showed a higher

amyloid deposition in anterior cingulate than slow converters. These findings would be in agreement with the idea that the hyperactivation observed in this brain region is due to the loss of inhibitory control, caused by the accumulation of AB (Garcia-Marin et al., 2009).

One of the main finding in AD's studies is the decrease in synchronization in high frequency bands, especially in alpha (Berendse et al., 2000; Locatelli et al., 1998), so the hypersynchronization usually observed in pMCI patients seems to be paradoxical. However, the synaptic disruption produced in the cholinergic system (Requena, Maestú, Campo, Fernández, & Ortiz, 2006; Schliebs & Arendt, 2006), and the loss of inhibitory neurons caused by the accumulation of neuritic plaques (Garcia-Marin et al., 2009) may produce the increase in synchronization in high oscillations in these patients. The synchronization found in the alpha band and in the occipital cortex is consistent with eyes closed resting state condition. Occipital lobe has usually been considered less impaired or not prominently involved until the last stages of the AD, but there is evidence of amyloid pathology in MCI patients and even in healthy subjects (McKee et al., 2006; Uhlhaas et al., 2008). Thus our results denote that occipital lobe appears to be part of a disrupted network which reflects that pMCI patients present a higher amyloid pathology than sMCI subjects.

In line with previous studies, pMCI patients showed a worse performance in episodic memory, achieving this ability the higher classification rates (M. Albert, Moss, & Blacker, 2007), but also in semantic memory and executive functioning. These findings suggest that extra-memory cognitive impairments may also have a role in the prediction of progression from MCI to AD (Chapman et al., 2012). In addition, the hypersynchronization exhibited by pMCI patients was inversely correlated with MMSE scores, Immediate Recall and Semantic Fluency, indicating that it has a negative effect on memory and general cognitive status of patients.

At structural level, there were no differences in both hippocampal volumes between sMCI and pMCI patients (Peters et al., 2014), although left hippocampus was slightly smaller in the pMCIs. These results could explain the differences obtained in verbal memory between both groups. Although the hippocampal atrophy is one of the anatomical changes that predict the subsequent conversion to AD (Clifford R Jack et al., 1999) it should be pointed that in AD patients, functional alterations usually precede structural changes (Small, 2005). In fact, the increase in synchronization showed by pMCI subjects was negatively related to both hippocampal volumes, pointing out that this increment reflect higher brain atrophy in one of the more vulnerable structures of AD. Finally, the higher classification scores obtained in the hypersynchronized links separately or combined with neuropsychological information suggest

that MEG functional network organization is able to detect the functional changes caused by synaptic disruption that precede the structural ones. This result points out the MEG as a promising biomarker of synaptic disruption in the prediction of the AD.

## **GENERAL DISCUSSION**



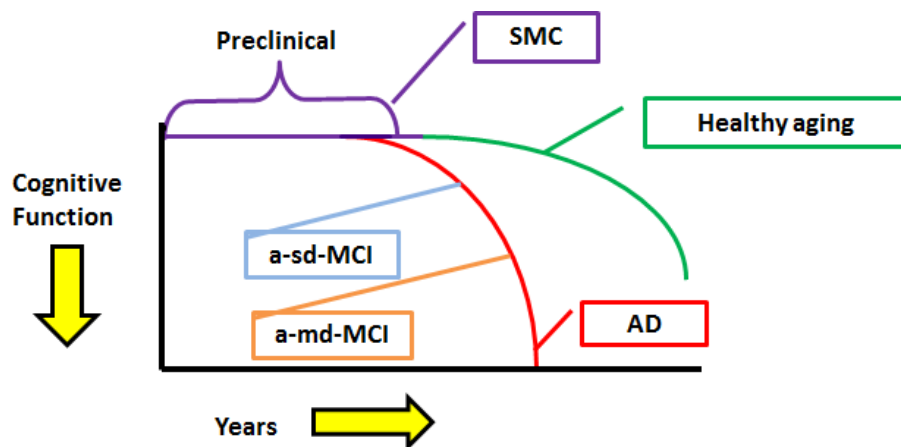
## MAIN FINDINGS

The principal aim of this thesis was to explore the biomagnetic patterns of brain activity in the intermediate stage between normal aging and the diagnosis of clinically probable AD, described as Mild cognitive impairment (MCI). For this purpose, a non-invasive neurophysiological technique was used (MEG) during two cognitive conditions: resting state and Internally Directed Cognitive State (IDICS). In order to achieve a comprehensive and complete picture of this clinical entity, MCI patient's brain activity/connectivity was related to structural damage (i.e. hippocampal atrophy) and cognitive status in two cross-sectional and one longitudinal study.

The main findings of this thesis may be summarized in six main points:

### 1. Healthy controls and MCI patients (a-sd-MCI and a-md-MCI) during resting state

Based on the model proposed by Sperling et al. (2011), our findings would support the concept of dementia as a continuum and could fit as follows:



**Figure 1.** A modified model of the continuum of the AD proposed by Sperling (2011). In the preclinical stage (purple), we would find Subjective memory complaints (SMC), among other cases (e.g. asymptomatic individuals with biomarker evidence). In the clinical state (red), cognitive function would decrease progressively until the appearance of the dementia. During this continuum it would be expected that a-sd-MCI (blue) would precede a-md-MCI (orange) to end with AD (red). It should be pointed that not all the cases of the preclinical and clinical stages would develop AD, since there are subjects who revert to normal state, and others that remain stable.

Considering the preclinical stage as a completely asymptomatic period in which there are not objective deficits by standardized measures, but subjective decline from previous level of memory functioning could be referred (Vestberg, Passant, & Elfgren, 2009), SMC could perfectly fit in this phase. It is estimated that between 17% and 57% of elderly people report them (Ganguli et al. 2004; Jessen et al. 2007; Mitchell 2008a), and that its percentage increases with age, rising to 43% for those aged 65–74, 51% for those aged 75–84, and 88% for those aged 85 and older (Bassett and Folstein, 1993). Although SMC have been related to depression, anxiety and personality traits (Jonker, Geerlings, & Schmand, 2000; Jorm et al., 2004), there is a growing evidence which relate them to an increase risk of develop dementia and also with the presence of biological correlates of early Alzheimer's disease (Rodda, Dannhauser, Cutinha, Shergill, & Walker, 2009).

SMC are usually present in the following stage, amnesic MCI (Petersen 2004; Mitchell 2008b). But in this case, there is an objective decline in cognitive functioning that primarily affects episodic memory (Petersen 2004; Gauthier et al., 2006). According to our results from the Study I, a-sd-MCI and a-md-MCI could not been considered as two independent entities but rather as two progressive states, being a-md-MCI a more deteriorate state than the a-sd-MCI and therefore, closer to AD. Although they did not differed in hippocampal volumes, the a-md-MCI presented more cognitive areas affected and a “slower” spectra pattern than a-sd-MCI, being these changes more evident when compared to healthy controls. These findings suggest that both amnesic subtypes of MCI firstly exhibit memory problems, and then as the disease progresses, the deterioration become increasingly global, such as in AD.

Regardless the subtype of aMCI, the connectivity results from the Study II would also support the proposal of dementia as a continuum. Brain is composed by localized but interrelated specialized areas, whose collaboration is essential to achieve an appropriate cognitive functioning. When a disruption occurs in these connections, dysfunction appears, producing the so-called “disconnection syndrome” (Catani & Ffytche, 2005). Therefore, connectivity measures allow exploring the functional relationships between different brain regions, and would be suitable to check if MCI could be also considered as a “disconnection syndrome”. In our results, MCI patients exhibited a decrease in high frequency bands (alpha and beta), an increase in low frequency bands (delta and theta) and a different pattern of synchronization in gamma band than controls. All these hyper and hypo synchronizations observed in the MCIs, which affected both interhemispheric and anterior-posterior connections, were associated with: 1) hippocampal atrophy; 2) a worse performance in memory, attention, language or executive functioning and 3) a more impaired global cognitive status (i.e. MMSE). These

findings suggest that in MCI would be taking place a malfunctioning along with a beginning of disconnection of the brain networks. Both phenomena would precede the “disconnection syndrome”, concept proposed to explain the AD (Delbeuck et al., 2003), in which a total disconnection occurs as the disease progresses.

A possible hypothesis to explain the slowing of background activity, along with the loss of functional connectivity and cognitive alteration in MCI subjects is the cholinergic deficit (Ebert & Kirch, 1998; Haense et al., 2012). The loss of neurons in the nucleus basalis of Meynert is related to the decrease of the neurotransmitter acetylcholine (Mufson et al., 2000), which is a cortical excitatory modulator (Francis, Palmer, Snape, & Wilcock, 1999). This approach relies on the increment of delta and theta activity and on the decrement of alpha and beta power (Kikuchi et al., 1999; Osipova et al., 2003; Sannita, Maggi, & Rosadini, 1987); along with a decrease in coherence in all frequency bands (Sloan, Fenton, & Standage, 1992; Kikuchi, Wada, Koshino, Nanbu, & Hashimoto, 2000; Osipova et al., 2003) after the administration of scopolamine (a cholinergic antagonist) in healthy subjects. A recent study conducted by our group with PLV found similar connectivity results and also architecture of the network in the alpha band comparable to that of AD under scopolamine’s condition (Stam et al. 2009; Bajo et al., 2014).

In addition, animal studies described an increase in low frequency coupling and a reduction in high frequency non- linear coupling as a result of the loss of acetylcholine (Villa et al. 2000). Moreover, it should also be noted that exists a bidirectional relationship between cholinergic neurotransmission and amyloid deposition during the disease (see Schliebs & Arendt, 2006 for a review). All these findings would help to understand, at least partially, the neuropsychological changes observed in MCI patients.

## **2. Differences in brain connectivity during the performance of a cognitive task between controls and MCI patients. What does it mean?**

Different EEG and MEG studies which used a memory paradigm to investigate how MCI's brains work, found an increase in synchronization in all frequency bands analyzed and also a distinct topography of connectivity between patients and controls (Pijnenburg et al. 2004; Jiang et al. 2008; Maestú et al. 2008; Bajo et al. 2010; 2011). Results from Study II are in line with these findings, despite of being a different cognitive task, since the MCI group exhibited a higher interhemispheric connectivity in delta, theta, beta and gamma bands. However, the internal nature of the paradigm used in this thesis could be the reason for the discrepancies obtained in the alpha band.

These rises of interhemispheric connectivity have been usually interpreted as a compensatory mechanism in order to overcome the inefficiency of the networks which are needed to perform a specific task. Namely, the recruitment of additional networks is required to achieve the same performance level than a control group. This explanation could be plausible for our results, since MCIs and controls executed the task at the same level, but with higher levels of brain synchronization in MCIs. However, if this compensatory mechanism would be enough to achieve results as good as control's, why MCI patients showed worse scores in neuropsychological tests? And also, why these increases in synchronization were inversely correlated with all these cognitive measures?

It seems that the increment in connectivity in delta, theta, beta and gamma bands is the way that MCI patients have to supply the lack of alpha synchronization during the task. And thanks to this increase, MCI subjects were able to perform the 1-subtraction task as well as controls. This way of compensation would be focused on the interrelation of the different frequency bands and would allow the execution of very simple tasks. Nevertheless with the increase of the difficulty of the task (3-subtraction), this connectivity starts to fall in all these frequency tasks, without affecting the execution yet. Although more levels of difficulty of the task would be needed to check if synchronization levels would continue to fall and the execution would finally be affected, the hypersynchronization observed in the MCI group, could be interpreted as a failed attempt of compensation (which only allows to execute the simplest tasks, and for this reason MCIs patients do not achieve the same level of execution in neuropsychological tests than the controls) or as a characteristic pattern of neuronal hyperexcitability that takes place during the pathological process of AD (since this excess in connectivity is related to a

worse performance in many neuropsychological tests and also to more deteriorated cognitive state).

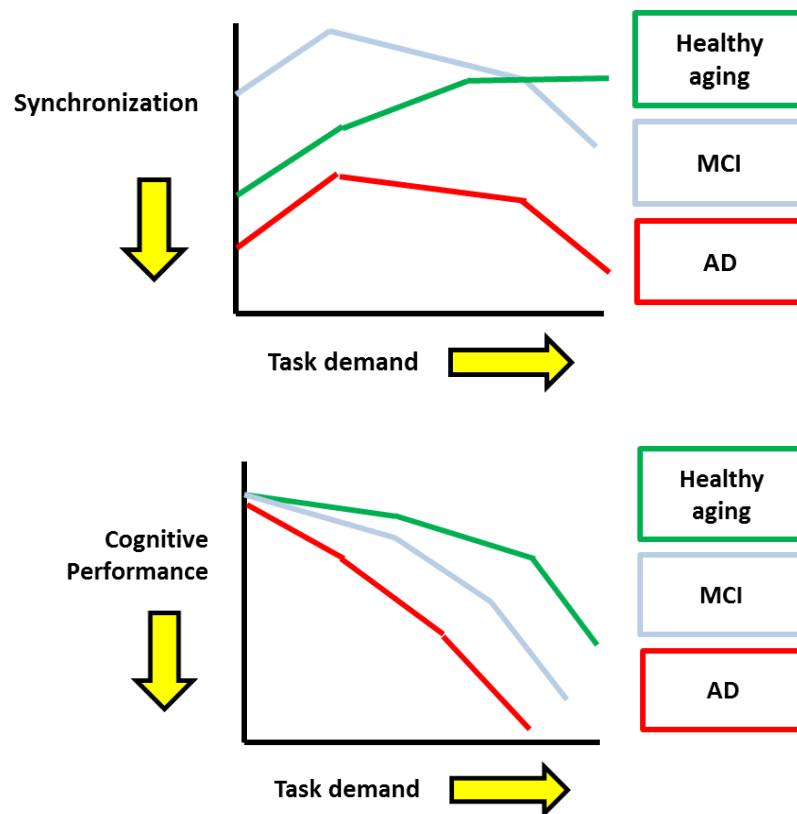
### **3. The increase of task demand in controls and MCI patients**

Contrary to our results, previous studies have described higher values of inter and intrahemispheric coherence in MCIs than in controls with the increase of task difficulty (Jiang and Zheng 2006; Zheng et al. 2007; Jiang et al. 2008). However, the failure to find differences in the resting state between both groups in those studies could be an important reason to explain these discrepancies.

Therefore, we considered that the results obtained in the Study II could be explained with the model proposed by Reuter-Lorenz & Cappell (2008), the compensation-related utilization of neural circuits hypothesis (CRUNCH). This theory considers that at the same level of task demand, old subjects show higher levels of activation than youngers, in order to achieve the same accuracy. However, when the task load increases, old subjects reach a resource ceiling before youngers, causing a decrease in their activation levels and subsequently in their performance.

In our case, controls exhibited mainly higher levels of synchronization under the 3- subtraction condition in delta band, and higher levels of desynchronization in beta and to a lesser extend in the gamma band. While other frequency bands remained more or less stable. MCI patients showed higher levels of synchronization in all frequency bands during the 1- subtraction condition, except in alpha. Nonetheless, their connectivity values were dismissed during the 3- subtraction task, although their performance was not significantly different from the controls. These results point out that not only the activation or synchronization of specific brain areas is needed to perform a cognitive task. But also the deactivation or hypo synchronization of others is equally important to perform a task properly, since the suppression of irrelevant regions allows inhibiting unimportant information or redirect those resources that are really needed (Wermke, Sorg, Wohlschläger, & Drzezga, 2008).

According to the CRUNCH model, the previous studies and our results, a model of synchronization during the execution of cognitive tasks could serve to hypothesize what would happen in the continuum of dementia. In order to explain this model, two schemes focused on the increase of synchronization have been proposed (for desynchronization the relation would be opposite): The patterns of synchronization would be represented in the top and the performance level, at the bottom.



**Figure 2.** A model of synchronization during the execution of cognitive tasks in the continuum of dementia

Under this approach, an increase in synchronization according to the task demand would be expected in the control group, until reaching their ceiling and their performance would start to drop. So, the healthy old subjects (in green colour) would be the “ideal” model of synchronization for dementia. Nevertheless, the MCI group (in blue colour) would start showing a hypersynchronization with low task demand and when the difficulty slightly increase, their levels of connectivity would begin to decrease, causing a lower level of execution. Finally, AD patients (in red colour) would start with lower levels of synchronization than the other groups, showing at low demand task evident execution problems. Inasmuch as the difficulty of the task enlarges, AD subjects would show a decrement in connectivity until they consequentially would not able to perform the task.

#### **4. ICICS and the role of alpha band**

In Study II, a new conception of cognitive paradigm was applied. Normally, neuroscience experiments use external stimuli in their experiments to evaluate the cognitive status of the participants. Besides in normal and pathological aging, cognitive studies are usually focused in external tasks (Caravaglios, Muscoso, Di Maria, & Costanzo, 2013; Clément & Belleville, 2012; F Maestú et al., 2008). The concept of “Internally Directed Cognitive State” (IDICS) surged out of a curiosity to explore how brain works during a purely internal task. Therefore, a cognitive function which is usually impaired in AD, calculation, was used in an internal way. This task needs the knowledge about numbers and arithmetic (long term memory), the ability to manipulate and update the information mentally (working memory), self-monitoring and the capacity to inhibit the distracting stimuli (executive functioning). Its easy adaptability and application, along with the interesting information that provides, makes this paradigm a good candidate to study internal processes in different populations.

Which role plays alpha during IDICS’s execution? It is well known that alpha is suppressed (desynchronized) during eyes open (Berger, 1929), and during the execution of a variety cognitive tasks (see Klimesch 2007 for a review). However, in our study, the control group showed an opposite pattern, that is, an interhemispheric increase in synchronization while performing the task.

In the early research about the alpha band, its synchronization was considered as a reduction in information processing (Pfurtscheller, 2001), or even as a “cortical idling” (Pfurtscheller, Stancák, & Neuper, 1996). However, latest empirical evidence also leads to interpret the alpha synchronization as an inhibition process (see Klimesch et al. 2007 for a review), since several studies have found this increment under internally directed attention, working memory and top-down processing (Benedek et al., 2011; Cooper et al., 2003; Jensen et al., 2002; Sauseng et al., 2005). This last hypothesis about alpha synchronization may explain the results obtained in the control group because it seems that an interhemispheric synchronization was necessary to execute it correctly. In addition, this increase in synchronization could reflect the inhibition of those brain areas which were not required for the task (Klimesch 2007), allowing the involvement of the relevant ones, such as the prefrontal areas.

However, in the MCI group the alpha band remained more or less stable during both resting and task, while other frequency bands exhibited a remarkable change between the two conditions. These results may suggest that in MCIs the inhibition system is being altered as consequence of the disease, and other frequency bands try to balance this impairment to deal with IDICS. The accumulation of amyloid plaques in the brain causes a decrease in GABAergic

neurons (Garcia-Marin et al., 2009) producing a hyperactivity of the pyramidal neurons and therefore, an overall system-wide hyperexcitation. This approach could explain the hypersynchronization observed in most of the frequency bands in MCI patients and the lack of synchronization in alpha, which may reflect the process of inhibitory control that is needed to perform an internal cognitive task such as IDICS.

## **5. Conversion from MCI to AD**

Study III is the first longitudinal investigation of synchronization brain patterns in source space in MCI patients. It should be pointed that the average of progression of our sample is approximately of one year, so pMCIs may be considered as “faster” converters. The exhaustive cognitive examination that our MCI patients underwent in the three studies, has provided evidences about the importance of the impairment in other cognitive domains beyond episodic memory to predict which MCI patients will finally develop AD. On the other hand, as in Study I, there were no differences in hippocampal atrophy between subtypes of MCI (in this case between pMCI and sMCI patients), but in neurophysiological patterns. These findings demonstrate that neurophysiological information is very useful to detect functional changes that often precede structural changes making it an essential tool in the study of neurodegenerative diseases, such as AD.

Previous connectivity EEG/MEG studies in the sensor space found an increase of connectivity in pMCIs mainly in high frequency bands (Rossini et al., 2008; Bajo et al., 2012), which is consistent with our results. As in most resting state studies there is a decrease in synchronization in alpha and beta bands in AD and also in MCI patients (see Study II), compared to controls, these results seem to be contradictory. However, if we consider the AD as a dynamic process and the hypothesis about the loss of inhibitory synapses due to the presence of amyloid plaques (García-Marin et al., 2009), the hypersynchronization observed in our pMCIs may be explained by an increment of accumulation of AB plaques in anterior cingulate and temporo-occipital cortex. If we would compare our sMCI patients with slow converters (i.e. 5 years), we surely would get a different pattern of synchronization depending on the areas that would be most pathologically affected in those patients. Therefore, as the disease progresses, the hyperexcitability of the pyramidal neurons would lead to neuronal death causing the desynchronization observed in more advanced stages of the AD.

Besides, the increase in synchronization observed in pMCI was inversely correlated to hippocampal volumes and neuropsychological performance, suggesting that it is a



manifestation of a pathological status. In Study II also the changes observed in synchronization in the MCI group would fit with this interpretation instead of with the compensatory hypothesis. It would be interesting to explore the connectivity patterns of the pMCI patients while performing the IDICS task to get a better understanding about how the disease disrupt the brain integrity of those that finally convert to AD.

## **6. The role of MEG in AD research**

The early detection of AD is one of the biggest concerns of clinical practice and research field because provides clarity to patients and caregivers, allowing a planning of their future; and allows the immediate beginning of pharmacological and cognitive therapies that may help to slow down the progression of the disease.

The new diagnosis criteria proposed by Dubois is based on clinical and biological evidence (MRI, PET or CSF). However, the neurophysiological information supplied by EEG or MEG have not been already considered as a biomarker. It should be pointed that existing biomarkers reflects the pathological but not necessarily the clinical state of the patients (Shaw et al., 2009), since organic alterations usually start some years before the onset of symptoms (Aizenstein et al., 2009).

MEG is a non-invasive technique that measures the neuronal activity directly, whose easy and relative fast application helps to be a “patient friendly” technique. Besides it can be repeated as often as necessary, making it an appropriate tool for monitoring the progress of the patients.

The use of this technique in the AD research have reported additional data for both diagnosis and prognosis of MCI and AD patients, since it provides information about synaptic disruption, which is a key feature in this disease (Zamrini et al., 2011). Our results would support this proposal, as it has provided relevant knowledge about the differences in brain activity/connectivity patterns that exist between MCI patients and healthy controls in both resting and cognitive states, and also about those MCI subjects who finally developed AD. Furthermore, its relationship with neuropsychological and anatomical evidence along with its ability to detect functional changes that precedes the structural ones (Small, 2005) indicates that MEG seems to have a clinical potential. Lastly, the chance to choice different types of analysis of the MEG data and the evidence that MCI-related studies show similar results indicate the necessity and utility of using complementary information to improve the understanding of the problem. The methodological approach would depend on the hypothesis

and the main issues that the researchers try to answer, and nowadays its fast development indicates the versatility that MEG data may provide.

Future research should focus on combining the MEG signal with the information obtained from different techniques in order to check and validate its diagnostic and predictive capacity, to be considered as a biomarker in the EA criteria.

## **CONCLUSIONS**

### **Study I**

- Both aMCI subtypes showed a slowing of the background activity in comparison to healthy controls, which was related to a lower hippocampal volume and a worse cognitive status.
- The spectral pattern of a-md-MCI patients, compared to the a-sd-MCI's one, seemed to be closer to the typical AD profile, and was also associated with a more severe cognitive deterioration.

### **Study II**

- In resting state, the MCI group exhibited a desynchronization in high frequency bands and a hypersynchronization in low frequency bands compared with the control group. Besides, this pattern was correlated with higher hippocampal atrophy and cognitive deficits.
- During the execution of the IDICS task, MCI patients showed higher synchronization values in all frequency bands except on alpha band, which were associated with lower scores in neuropsychological test and hippocampal volume. These results suggested MCI subjects may present an inhibition problem.
- The CRUNCH model could explain the changes in synchronization caused by the difficulty of the task.

### **Study III**

- pMCIs showed higher synchronization in alpha band between right anterior cingulate and occipital and temporal areas, especially from the right hemisphere, than sMCIs.
- This increase was inversely correlated with the performance in several cognitive domains, suggesting that is the result of an aberrant effect caused by the pathology.

## **FUTURE DIRECTIONS**

From a clinical point of view, it would be interesting studying amnesic subtypes performing the IDICS task, in order to verify if there would be differences between them and also to examine if a-md-MCI patients would show lower levels of synchronization than a-sd-MCI group, as AD patients. Besides, the comparison of IDICS with a young group would help to understand how brain works without external stimuli and how the disease disrupts its functioning. The exploration of the DMN, which is one of the brain networks damaged in AD, during the execution of IDICS would bring us a better understanding of the nature of this network while performing an internal task. More MEG studies with different task load in normal and pathological aging would be needed to verify the synchronization's model proposed in the continuum of dementia. New approaches based on neuropsychological profiles instead of the diagnosis would be relevant to explore how the cognitive status changes the brain neurophysiological patterns. Furthermore, the study of the resting state as a dynamic process would allow us to characterize the brain activity over time and probably to make more accurate comparisons between patients and healthy elderly subjects. In addition, the combination of MEG and MRI, PET and CSF data may provide relevant evidence about the changes produced by the disease, and therefore MEG could be considered as a neurophysiological biomarker in a near future. To look into the evolution of the disease over time, different MEG recording of the same subjects should be compared and different variables such as genetics, cognitive reserve or gender should be explored. Moreover, the study of the effects of pharmaceutical and cognitive therapies would help to plan the future of the patients and their caregivers. Finally, healthy subjects with relatives with AD, biological risk or with SMC should be the next population to examine since their study would provide relevant clues about dementia.

From a methodological perspective, it would be interesting to analyze the MEG signal from the sensor space (Studies I and II) to the source location (Study III) with the aim to obtain more accurate information about the brain areas which would be involved. New approaches as graph theory may be applied both to resting and IDICS to explore the architecture of the functional networks and therefore, to better understanding of the MCI as a disconnection syndrome. Moreover, it would be necessary to examine the interaction among the different frequency bands to figure out how the disease alter this communication and cross frequency coupling could be a good tool to achieve it. Further studies should compare the different methodologies which are appearing in the literature in order to validate their reliability and thus, their robustness.

From a conceptual viewpoint, diagnosis criteria and etiological origin of the disease has been changing depending on the development of the neuroimaging and medical techniques. We are living a technological revolution which is modifying our way to understand AD. New hypothesis are emerging and demonstrating the complexity of this disorder, and therefore it is essential to integrate information which comes from different levels of analysis to propose an increasingly appropriate model of AD. For all these reasons, to advance it is necessary to be abreast of recent research and be able to adapt to the new proposals which are coming out in the study of AD.

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## **ANNEXES**